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INVESTIGATING THE EFFECT OF SUBSTRATE, MYCORRHIZAL APPLICATION AND BULB SEPARATION ON THE GROWTH OF THE WILD ORCHID *ANACAMPTIS PYRAMIDALIS*

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ABSTRACT

Pyramidal orchid (*Anacamptis pyramidalis*) is a wild terrestrial orchid widely found in Lebanon and the Mediterranean zone. Random collection and trade of the orchid for medicinal and edible use (salep) subjected it to a risk of extinction. Consequently, the current work aimed to propagate this orchid in vivo. Orchid bulbs were transplanted from the wild into four different soil substrates (Pinebark, Pinebark-Peat (1/1), Peat-Sand (1/1) and control: soil from the collection site) under controlled conditions of temperature and humidity. The effect of mycorrhizal application (MY: Yes) was tested in the different substrates except in control and orchid bulbs (son bulb and mother bulb) were planted combined (SB+MB) or after separation of the mother bulb (SB). Same treatments were repeated over two consecutive years. Results showed an earlier emergence of son bulbs (SB) grown in control substrate. Moreover, there was a significant difference in plant growth with superiority for (SB) compared to (SB+MB) regarding plant length and elongation of first leaf except bulb dimensions (length and width) that were higher in (SB+MB). Mycorrhizal application enhanced the overall growth of plants and its effect was the most obvious in the substrate Peat-Sand (1/1). In general, the best growth of the orchid was observed at the level (SB)/(MY:Yes)/Peat-Sand (1/1). Results of the second experimental year confirmed those of the first year with an improvement of the rate of emergence by 13%. The in vivo propagation method was beneficial on improving the growth of A. *pyramidalis* ex situ and it could be adopted as an initiative for wild orchid conservation.

Keywords: *Anacamptis pyramidalis, conservation, substrate, mycorrhiza, bulbs.*

INTRODUCTION

The Mediterranean region is one of the richest zones by its fauna and flora. Lebanon a part of this zone presents one of the best examples where a landscape and floristic diversity is found due to its geographical location and high variability

of abiotic conditions (Jomaa, 2008). Wild orchids are among this richness, where more than 87 species are found (Bou Dagher Kharrat, 2010), among which several species are considered as endemic in the east Mediterranean zone. The genus *Anacamptis* (Orchidaceae) consists of 11 species (Kretzschmar *et al*., 2007) and was first established by the French botanist Louis Claude Marie Richard (1754-1821) in 1817, based on *Anacamptis pyramidalis* (L.) Rich., the well-known Pyramidal Orchid (Wood and Ramsay, 2004). Pyramidal orchid is characterized by an erect stem, linear basal leaves, purple pink flowers in conical spike and ellipsoidal bulbs (Tohme and Tohme, 2014). In general, the Mediterranean terrestrial orchids including Pyramidal orchid have consistent annual growth pattern, beginning and ending by one or more dormant bulbs over summer during the drought period. Bulbs re-sprout in the following autumn and leaf formation begins in early winter and continues during winter cold season (Brundrett, 2014). This species is threatened under large scale collection pressure due to its economic value which requires the reproduction trials inevitably (Sevgi *et al*., 2012). It is strictly protected in some European countries such as Czech Republic and Slovak Republic where it is under the risk of extinction. It is simultaneously protected by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (Štajner *et al*., 2010). In Lebanon, *Anacamptis pyramidalis* is found in different regions like Quamouaa, Ehden, Boutmeh, Kfarhouna and others (Tohme and Tohme, 2014). Although this orchid is widely found in Lebanon, it is under extinction risk because of habitat alteration and random collection for edible or medicinal use.

Therefore, the main objective of this study was to provide a propagation tool for the pyramidal orchid by transplanting it from the wild in order to be grown under specific experimental conditions and to explore its behavior after it had been subjected to different growing techniques; like the type of substrate, mycorrhizal application and separation of its bulbs. Consequently, to study the effect of the different growing techniques and their interactions on the growth of the plant.

MATERIALS AND METHODS

Orchid collection

The behavior and growth habit of the wild orchid were assessed over 2 experimental years, 2014 and 2015. Pyramidal orchids were collected from the region of Wedeh el Karem-Mount Lebanon/Lebanon, situated at an altitude of 1140 m (33° 57' 0" North, 35° 45' 0" East), where they were found in a high density. In each experimental year, 140 plants were collected in early June during the flowering stage of the studied species (Lind *et al*., 2007). Entire plants were collected consisting of underground parts: a root system and two bulbs (a mother bulb (MB) that has already sprouted and given the inflorescence of the current season and a new bulb that will sprout in the next season after a dormancy period that was used in this study and referred to as son bulb (SB)) as well as aboveground parts (stem, leaves and flower). However, only bulbs were used as planting material.

Experimental design and treatments

The experimental design (Figure 2) included 14 treatments with 10 bulbs per treatment. Son bulbs were planted with or without the mother bulb ((SB) or (SB + MB)) in four types of substrates (Own soil, Pine, Pine+Peat and Peat+Sand) with or without mycorrhizal application (MY: Yes or MY: No). Own soil substrate represented the soil collected at a depth of 30 cm from the site where orchids were found. This substrate was considered as "Control". Pine substrate was formed by pieces of pine bark collected from the same site and cut into small pieces prior to use. Pine+Peat and Peat+Sand substrates were prepared by mixing peat with pine bark pieces and sand respectively in a ratio 1:1 in terms of volume. Substrates properties are represented in the following Table 1.

Table 1. Composition of different tested substrates

	Own Soil	Pine	Pine+Peat (1/1)	Peat+Sand (1/1)
Ph	7.57	5.96	5.77	7.51
EC ($mS.cm^{-1}$)	0.832	0.884	0.574	0.174
Organic matter (%)	4.6	76.3	84.3	7.3
Nitrogen (kjeldahl)(%)	0.6	0.6	0.67	0.326
P_2O_5 total (digestion) (ppm)	13.71	2771.77	424.61	67.09
K_2O total (digestion) (ppm)	375	1066.35	1531.46	588.61
CaO total (digestion) (%)	9.4	6.1	4.3	1.9
MgO total (digestion) (%)	1.4	0.7	1	0.3

The application of commercial mycorrhiza took place after bulb plantation in pots of 15 cm in diameter for all pots in various substrates except in the Own Soil substrate where mycorrhizal symbiosis was found naturally. Pots were put in a climate chamber where a constant temperature was maintained at 5°C during November, December and January, and then it was raised to 10^{o} C in February and to 15°C during March and April. Temperatures were fixed in a way to provide the optimal natural values during the growth of the pyramidal orchid in the wild. Substrates were continuously wetted by spraying water in order to preserve a high humidity level in the growing medium of bulbs.

Data recording

The experiment in both years was carried out over 6 months; from emergence to the end of the vegetative growth prior to flowering. Several parameters were recorded in order to evaluate and compare the emergence and growth of plants. The date of emergence was the number of days between transplantation date in first of July and the date of appearance of the first shoot tip at the soil level. The rate of emergence revealed the percentage of emerged bulbs among the total planted bulbs. The elongation of longest leaf was assessed by 3 readings (1, 2 and 3 months after emergence), and the stem length was assessed twice during the growing cycle (respectively 4 and 5 months after the emergence date). The timing of each reading was adopted in order to cover a part of the life cycle of *Anacamptis pyramidalis*,

starting by bulb emergence followed by leaf formation and ending with stem elongation. The experiment was stopped at this level in order to assess the bulb growth variation as affected by different factors. The bulb normally reaches its optimal growth prior to flowering. Therefore, bulb dimensions (length and width) were measured at the end of the experiment after plant removal from pots.

Statistical analysis

The effects of different factors (substrate types, mother bulb separation v.s. no separation and mycchorizal addition v.s. mycchorizal absence) and their interactions on the averages of the measurements were analyzed using Factorial ANOVA. For the elongation of the longest leaf and the plant length Repeated Measures Factorial ANOVA were applied to also study the time (readings) effect.

RESULTS AND DISCUSSION

Rate of emergence

The planted bulbs started to sprout and emerge above soil level after several months of dormancy. Some bulbs did not sprout. The emergence rates were 69 % and 82 % in the first and second experimental years respectively.

Date of emergence

Results showed a variation in the emergence period of potted bulbs (Figure 1). In the control substrate (Own Soil) an earlier bulb emergence was observed compared to other substrates. In this substrate and for both experimental years, the average date of emergence was higher in (SB+MB) than in (SB) (134 days and 130 days respectively). In general, for all substrates the mother bulb separation prior to plantation has led to an earlier emergence. Averages obtained in (SB) were lower than those in (SB+MB) in all substrates in case of no mycorrhizal application (MY:No) (135, 137 and 133 days in (SB) against 138, 142 and 139 days in (SB+MB) for the substrates (Pine), (Pine+Peat) and (Peat+Sand) respectively). Mycorrhizal application affected the date of emergence only in the substrate (Pine+Peat) in (SB) inducing an earlier emergence with a difference of 6 days observed between MY:Yes (131 days) and MY:No (137 days).

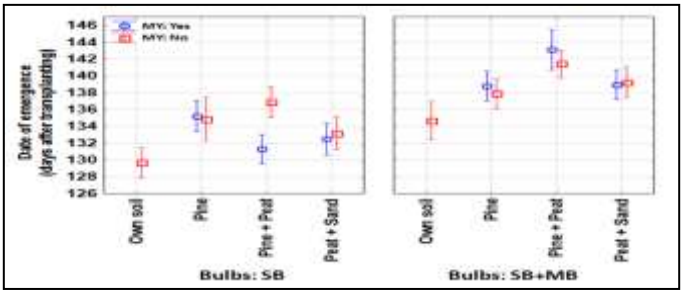

Figure 1. Variation of the average emergence under the effect of substrates type, mother bulb separation and mycorrhizal application in both years.

Elongation of longest leaf

This parameter was affected by the different investigated factors (Figure 2). The average elongation of longest leaf varied between substrates. For instance, in the second year experiment it was significantly the highest in the substrate (Own Soil) for (MY:No) in the final reading in February for (SB) and (SB+MB) with 23 cm and 22 cm respectively. There was a positive effect of mycorrhizal application on the elongation of longest leaf, in specific in the first year experiment for the substrate (Peat+Sand) with separation of mother bulb (SB), where an average of 20 cm was reached in (MY:Yes) compared to 15 cm in (MY:No). In addition, results showed that planting the son bulb alone (SB) has improved this parameter despite the mycorrhizal application. This was evident in the second year experiment, regarding the substrate (Peat+Sand) where a final average of longest leaf elongation of 16 cm was reached in (SB) compared to 8 cm in (SB+MB) although mycchoriza was absent (MY:No). Moreover, the combination of mother bulb separation (SB), mycchorizal application (MY:Yes) and (Own Soil) provided the best results with regards to this parameter; the highest average (24 cm) was recorded at the level (SB)/(MY:Yes)/(Own Soil) in the second experimental year.

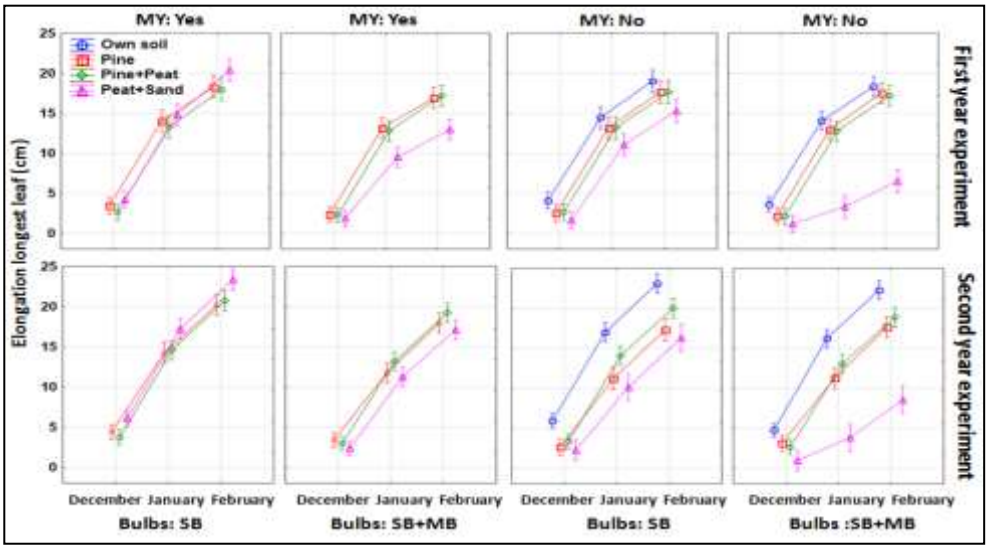

Figure 2. Averages (markers) and the 95% limits of confidence (vertical bars) of the elongation of longest leaf for the different levels of the experimental factors.

Plant length

The effects of mycorrhizal application and mother bulb separation showed an increase in plant length especially for this parameter especially when added to the effect of tested substrates (Figure 3). In case where bulbs were cultured without any additional treatment; no mother bulb separation (SB+MB) or no mycorrhizal application (MY:No), the control substrate (Own Soil) performed the best when compared to the other three substrates with a significant difference in the first

experimental year. The substrate (Peat+Sand) showed the lowest average for both experimental years; for (MY:No)/(SB+MB) the final average of plant length scored 14 cm and 20 cm respectively in the first and second experimental years. The mycorrhizal application gave effective results when tested alone without considering the effect of mother bulb separation. Its effect was the most obvious in the substrate (Peat+Sand) especially in the second year experiment where an increase of 20 cm was observed after mycorrhizal application for the level ((SB+MB)/(Peat+Sand)/(My:Yes)) where plants scored an average length of 40 cm compared to 20 cm for the same level without mycorrhizal application ((SB+MB)/(Peat+Sand)/(MY: No)). In addition, a positive effect of mother bulb separation was observed when tested alone without mycorrhizal application (MY:No). In the first year, in the substrate (Pine+Peat), the final average scored 34 cm in case of mother bulb separation while the average decreased to 29 cm in case of no mother bulb separation in the same substrate. Finally, plant length was influenced the most by the combination of mother bulb separation and mycorrhizal application in the substrate (Peat+Sand). For instance, in the second experimental year, the highest plant length was recorded at the level (SB)/(MY: Yes)/(Peat+Sand) with an average of 44 cm.

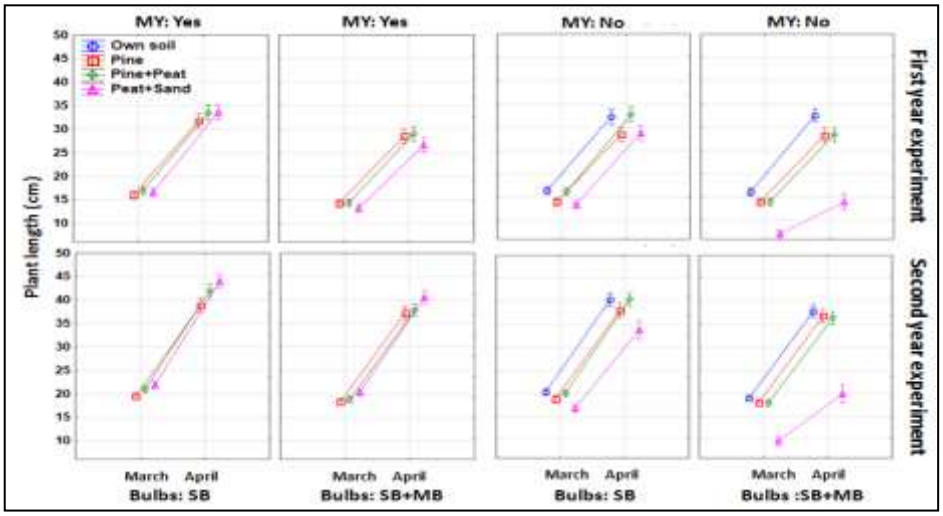

Figure 3. Averages (markers) and the 95% limits of confidence (vertical bars) of the plant length for the different levels of the experimental factors.

Bulb dimensions

It was found that bulb length and width were significantly higher in the second year experiment compared to the first year despite the effects of substrates, mother bulb separation and mycorrhizal application (Figure. 4). Mycorrhizal application enhanced bulb length and width and its effect appeared in all substrates despite the presence or absence of the mother bulb. In specific, it increased bulb length by 1.2 cm and bulb width by 0.3 cm in the level (SB+MB)/(MY:Yes)/(Peat+Sand)

compared to (SB+MB)/(MY:No)/(Peat+Sand) in the second experimental year. On the other hand, the effect of mother bulb separation on bulb dimensions differed according to the substrate type. On the other hand, the effect of mother bulb separation on bulb dimensions differed according to the substrate type. For instance, in the second year experiment the mother bulb separation affected negatively bulb dimensions in the control Own Soil substrate; the bulb length and width scored in the level (SB+MB)/MY:No/Own Soil an average of 3.4 cm and 1.3 cm respectively, while this average decreased after mother bulb separation in the level (SB)/MY:No/Own Soil and scored an average bulb length and width of 3cm and 0.9 cm respectively. For the Pine substrate the mother bulb separation did not have any significant effect especially for bulb length; in the second year experiment, the same average bulb length was obtained (2.6 cm) for both levels: (SB+MB)/MY:No/Pine and (SB)/MY:No/Pine.

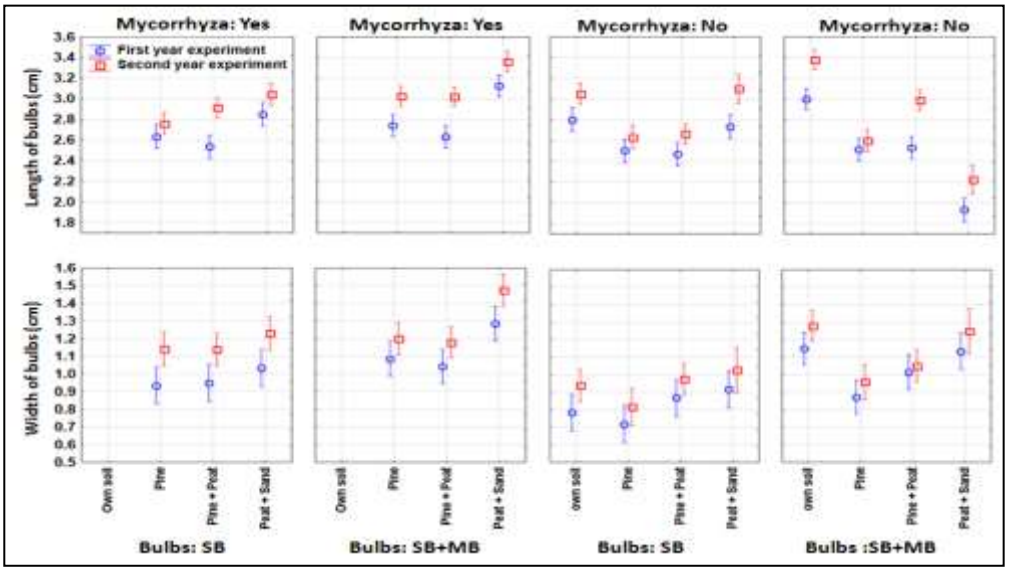

Figure 4. Averages (markers) and the 95% limits of confidence (vertical bars) of the bulb dimensions for the different levels of the experimental factors.

Anacamptis pyramidalis was successfully transplanted from the wild and grown under experimental conditions. The rate of emergence was improved by 10% when the mother bulb was kept and an earlier emergence of 3 to 8 days was observed when it was removed. This earliness observed for (SB) compared to (SB+MB) could be attributed to bulb maturity and its dormancy stage at the time of plantation. It seemed that the separation of the mother bulb have stimulated an earlier breakage of dormancy in the son bulb which might have caused an earlier emergence. On the other hand, planting bulbs in (Own Soil) while controlling temperature and humidity provided conditions of growth that were similar to the natural environment of bulbs in the wild. Microorganisms present naturally in (Own Soil) were absent in the other tested substrates which might have induced an

earlier date of emergence. Moreover, the positive effect of mother bulb separation and mycorrhizal application was obvious for the elongation of longest leaf and plant length in both experimental years regarding all substrate types except for the control (Own Soil) where mycorrhizal application was not adopted and where the mother bulb separation did not affect significantly the average stem length in both years. This could be related to the natural behavior of the Pyramidal orchid in its wild ecosystem. *Anacamptis pyramidalis* in the wild could reach a high length with one or even 2 bulbs. According to Sevgi *et al*. (2012) a length of 65.5 cm could be reached. The combination of mother bulb separation and mycorrhizal application enhanced the growth especially in the substrate (Peat+Sand) which was poorer in inorganic nutrients (N,P,K) compared to (Pine) and (Pine+Peat) substrates provided due to the fact that the beneficial effect of mycorrhiza is often associated with the low availability of inorganic nutrients (Dhillion and Friese, 1994). On the contrary, in case of mycorrhizal absence the high availability of organic matter and nutrients in the substrates (Pine) and (Pine+Peat) allowed plants to reach higher heights. Using (Own Soil) also appeared to enhance the elongation of the longest leaf compared with other substrates although they were richer in organic matter. This could be due to its higher pH (7.57) compared with those of (Pine) (pH=5.96) and (Pine+Peat) (pH=5.77) substrates. In fact, *Anacamptis pyramidalis* is found and prefers alkaline soil (Heinsoo, 2012), with a pH of 7.01 (Tsifsfis *et al*., 2008). Concerning bulb dimensions, mycorrhizal application enhanced the bulb growth while mother bulb separation had a variable effect depending on the substrate type. In addition, the (Own Soil) (Control) representing the natural substrate of Pyramidal orchid induced the best growth due to the adaptation of the bulb to this type of soil in nature. Finally, the mortality that happened on a relatively small percentage of bulbs could have been caused by some pathogenic factors in bulbs that could not be detected prior to plantation or to the normal difficulties that face the transplantation of a wild plant from the nature to be grown under experimental conditions.

CONCLUSIONS

Conclusions from this study were:

-Transplantation through different vegetative parts such as rhizomes, bulbs, and stolons is the simplest method for wildlife conservation compared to other conservation methods (cryoconservation, in vitro). Its adoption is recommended at local and national levels seeking to protect and conserve various wild species that could be threatened in degraded ecosystems or under risk of extinction.

-The success of in vivo experiments could provide a tool not only for conservation purpose but also for mass scale production.

-Re-introduction trials in the wild of ex situ propagated orchid plants could be investigated in future research studies.

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DIFFERENCE IN THE CARCASS QUALITY AND MEAT CHEMICAL COMPOSITION IN TWO LINES OF SLOW-GROWING CHICKENS WITH OR WITHOUT ACCESS TO PASTURE

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ABSTRACT

The study was carried out to compare the carcass quality and the chemical composition of breast and thigh meat in two lines of slow-growing male chickens - La Belle (LB) and Bresse Gauloise (BB) reared conventionally or outdoors, having access to pasture. The birds were slaughtered at 12 weeks of age. Two-way ANOVA was used to assess the effect of the rearing strategies as well as the line on the carcass quality and meat chemical composition. Rearing system affected significantly the carcass traits of the birds. The lines reared on pasture had lower live and carcass weight as well as lower dressing percentage ($P<0.001$). On the other hand they had higher percent ($P<0.001$) of the edible by-products (neck and giblets). The percentage of the breast meat was significantly reduced in the birds that had access to pasture ($P<0.001$), while thigh remained unaffected. The pastured chickens displayed higher part of the wings ($P<0.01$). Outdoors rearing influenced the chemical composition in the breast and thigh meat of the lines. Chickens reared on pasture were characterised by lower lipid content ($P<0.01$) in breast and reduced protein in thigh ($P<0.05$), as well as increased moisture in both kinds of meat, however depending on the line ($P<0.05$). Furthermore, pasture access resulted in reduced ash content of the breast meat ($P<0.001$), which was lower in the LB chickens ($P<0.01$), while in thigh this parameter was strongly determined by the interaction of the rearing system and line of the birds ($P<0.01$).

Keywords: *Slow-growing lines, carcass, meat, indoors rearing, pasture access.*

INTRODUCTION

In recent years, the outdoors rearing systems have gained much attention due to the increasing consumer demands for natural poultry products. It is known that chickens grown conventionally experience higher stocking density, increased levels of stress and hence are more susceptible to diseases (Doziers *et al*., 2005; Lin *et al*., 2006), as these factors generally decrease the quality of the meat. Hence, one of the main expectations of the outdoors rearing is to increase the safety, as

well as the nutritional and healthy value of the poultry meat. So far, studies report influence of the outdoors systems on the quality attributes of poultry meat, however the findings are inconsistent, and mostly dependent on other factors. Fanatico *et al*. (2005) observed more tenderness in outdoors reared chickens when compared to the indoors grown, but differences were also attributed to effect of the genotype and sex. Yang *et al*. (2015) reported decrease in the drip loss of the meat of free-range reared birds but increased shear force that resulted in tougher meat. On the other hand, Ponte *et al.* (2008) recorded higher overall acceptability including tenderness in the meat of free range reared chickens with pasture access.
The outdoors rearing with pasture access is very similar to the natural environment with positive influence on the welfare of the birds. This however, is associated with different levels of pasture intake and might affect negatively the performance in fast growing genotypes due to decreased weight gain and feed efficiency. Furthermore, as shown by Mancinelli *et al*. (2017), the fast growing lines may be less adaptive to outdoors rearing, which makes such practices suitable for the slow – growing lines. Two such lines are used in this study- the autochthonous La Belle - representative for the national gene pool in Bulgaria, as well as the old French Bresse Gauloise. Research on these lines concerning their carcass and meat quality are relatively few (Popova *et al*., 2016; Popova *et al*., 2017; Popova *et al.*, 2018). However, based on the results so far, these lines draw much interest in finding possibilities and best rearing practices for raising slower-growing chickens to produce high quality meat. Hence, the aim of this study was to evaluate the differences in the carcass traits and meat chemical composition in these two lines of slow-growing chickens, as affected by the conventional rearing or pasture access.

MATERIAL AND METHODS

Experimental birds and rearing systems

The experiment was designed as two trials that were carried out respectively in the experimental poultry farm of the Institute of Animal Science–Kostinbrod, Bulgaria (conventional rearing) and Livadi symbiotic farm located in Damyanitsa village, Bulgaria (pasture rearing) with male slow-growing chickens of the two lines La Belle (LB) and Bresse Gauloise (BB). For the first trial, a total of 73 LB and 51 BB 1-day old male chickens obtained from the parent stock in the Institute were placed into a deep litter facility with a stocking density of 14 birds/m^2 in separate pens but in the same poultry house in the Institute. All the birds were fed ad libitum starter (ME - 13.18 $MJ.kg^{-1}$; protein content - 19.41%) and finisher (ME - 13.00 $MJ.kg^{-1}$, protein content - 17.77%) for 4 weeks and 8 weeks, respectively. Water for the chickens was provided ad libitum with a nipple drinker. The lighting regime was 15 h of light and 9 h of darkness, and the temperature ranged between 20 and 24℃ (started from 32-36℃ in the first 3 days after hatching and decreased afterwards). For the second trial, the total number of male chickens reared in Livadi farm was 48, divided into two groups, each containing 21 and 27 chickens according to the line – LB and BB. The birds were reared in controlled

microclimate conditions until they reached 3 weeks of age (as described by Salatin, 1998). From 4 to 12 weeks of age, the chickens were reared in wooden cages covered inside with aluminium plates to prevent the overheating. The cages were equipped with nipple drinkers and feeders while being open so that the birds could have access to pasture. Additionally, the chickens were fed ad libitum the same diet as the ones from the trial in the Institute.

Carcass composition

At 12 weeks of age, 6 birds of each line from both trials (rearing systems) were selected for slaughter based on the average live weight. After stunning, decapitation and bleeding, the carcasses were plucked, eviscerated and their feet removed. The edible by-products (neck, liver, gizzard, heart and spleen) were weighed and their content was calculated as percentage of the live weight. Hot carcass weight was recorded and dressing percentage was calculated. The carcasses were then stored at 4^0C for 24 h and weighed again. Further the internal fat was removed from the carcasses and they were separated into breast, thigh, back and wings. The weight of the internal fat and the parts was recorded. The skin and bones from the breast and thighs were removed to obtain the muscles and they were also weighed. The content of the separated parts, muscles and internal fat was calculated as percentage of the cold eviscerated carcass weight. Then the muscles were minced and frozen at -20 ^{0}C until further analysis of the chemical composition of the meat.

Meat chemical composition

The breast and thigh meat was analysed for lipid, protein, moisture and ash content following the AOAC 2004 Official method of analysis.

Statistical evaluation

The data were statistically evaluated by two-way ANOVA as the line of the birds, the rearing system and their interaction were included in the model. The JMP v.7 software package was used to perform the statistical analysis (JMP Version 7, SAS Institute Inc. Cary, NC).

RESULTS AND DISCUSSION

Carcass traits

As presented in Table 1, regardless of the line, the chickens that had access to pasture exhibited significantly lower live weight at 12 weeks of age ($P<0.001$), when compared to the conventionally grown. Consequently, the carcass weights were also considerably lower in the pastured lines ($P<0.001$), as was the dressing percentage ($P<0.001$). Different studies have examined the conventional rearing or pasture access in chickens, however, the effect of the rearing system on the live weight of the birds remains inconclusive and dependent on other factors such as chicken genotype and sex. Sogut *et al.* (2011) reported considerably lower live weight in broilers reared organically with access to pasture when compared to conventionally grown ones. Similarly, Poltowicz and Doktor (2011) showed that free range with pasture decreased the live weight in the chickens, but only in the male ones. On the other hand, Cömert *et al*. (2016) observed significantly higher

live weight in fast-growing chickens, reared outdoors, but no effect of the rearing system in slow-growing lines. Our results are in line with Wang *et al.* (2009) who reported lower live weight in slow- growing chickens reared free-range with access to grass paddocks and Li *et al*. (2017) in medium –growing chickens raised indoors on floor and outdoors with pasture. However, both studies did not observe any significant difference between the free-range and conventional system in regard to the dressing percentage. Despite that no significant difference was found in regard to the carcass weight, Fanatico *et al.* (2008) reported lower ready to cook carcass yield in slow-growing chicks reared outdoors which coincides with our results. On the other hand the same study did not report effect of the rearing system on this trait in fast growing genotype.

Table 1. Live weight, carcass weight, dressing percentage, edible and inedible parts, and abdominal fat deposition in La Belle and Bresse Gauloise chickens, reared conventionally or with pasture access

Item	Conventional		Pasture access		S.E.	Significance of the factors		
	LB	BB	LB	BB		Rearing system	Line	Rearing system x Line
Live weight/ g	1986.67	1973.83	1317.66	1370.66	52.01	***	NS	NS
Carcass weight (hot)/g	1235.33	1201.16	794.66	818.33	52.06	***	NS	NS
Carcass weight (cold),g	1211.83	1172.50	748.00	740.00	56.01	***	NS	NS
Dressing percentage, %	62.18	60.86	56.80	53.93	2.38	***	NS	NS
Inedible parts, %	12.19	12.01	14.66	13.33	1.00	***	NS	NS
Neck, %	2.01	2.18	2.79	2.88	0.25	***	NS	NS
Liver, %	1.87	1.89	3.12	3.26	0.32	***	NS	NS
Gizzard, %	1.84	2.12	2.43	2.42	0.29	**	NS	NS
Heart, %	0.53	0.54	0.67	0.66	0.08	***	NS	NS
Spleen, %	0.17	0.18	0.22	0.20	0.06	NS	NS	NS
Edible, %	6.42	6.91	9.42	9.53	0.55	***	NS	NS
Abdominal fat, %	2.37	2.58	2.44	1.94	0.96	NS	NS	NS

S.E.- standard error; ** P<0.01; ***P<0.001.

The content of the inedible parts were higher in the birds reared on pasture (P<0.001), and the same influence of the outdoor access was observed in regard to the percentage of the neck (P<0.001), liver (P<0.001), gizzard (P<0.01) and heart (P<0.001) in both LB and BB lines. The significantly increased content of the internal organs, especially those involved in the digestion that we observed in this study in the birds reared outdoors could be associated with the pasture and consequently the higher fiber content which stimulated the development of the gastrointestinal tract. In line with this statement, Dou *et al.* (2009) showed higher percentage of the stomach in chickens reared free-range with access to grass. On the other hand, contrary to us, Bartlett *et al*. (2015) did not find any significant difference between conventionally and pastured broilers for the content of their heart, liver and gizzard, however they recorded decreased intestines in the pastured chickens.

One of the major goals in the poultry meat industry is to reduce the carcass fatness and mainly the deposition of the abdominal fat (Fouad and El-Senousey, 2014). Jiang *et al*. (2011) and Li *et al.* (2017) found significant decrease in the abdominal

fat as a result of the pasture rearing, while Cömert *et al.* (2016) observed generally increased abdominal fat in outdoor reared lines, more pronounced in the fast growing ones. No effect of pasture was detected on the percentage of the deposited abdominal fat in our study, which is in agreement with the results of Mikulski *et al*. (2011) and Chen *et al.* (2013).

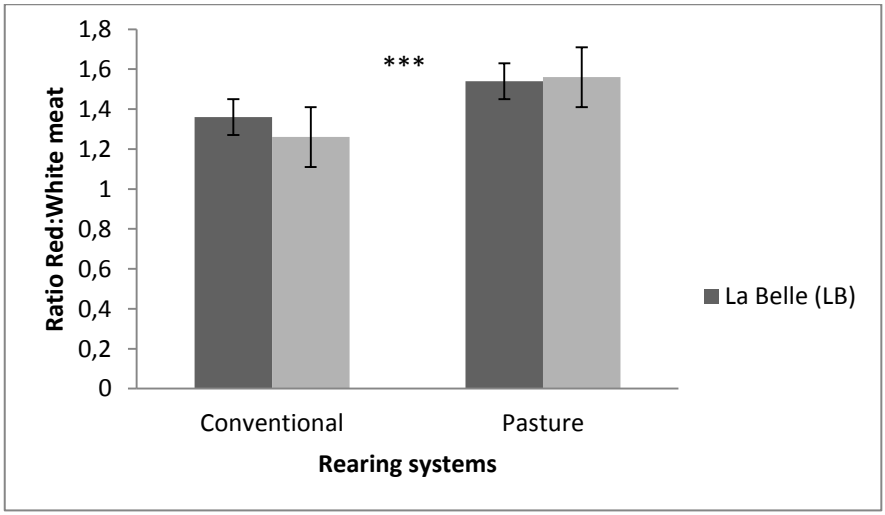
While there were no differences between the lines in regards to the main carcass parts, the latter were affected to a great extent by the pasture access of the birds (Table 2).

Table 2. Main carcass parts in LB and BB lines reared conventionally or with pasture access

Item	Conventional		Pasture access		S.E.	Significance of the factors		
	LB	BB	LB	BB		Rearing system	Line	Rearing system x Line
Breast(skin+bone), %	27.09	29.04	26.24	25.96	1.56	**	NS	NS
Breast (muscle), %	18.10	19.60	15.44	15.76	1.38	***	NS	NS
Thigh (skin+bone), %	37.34	36.09	36.71	37.07	1.59	NS	NS	NS
Thigh (muscle), %	24.62	24.49	23.69	24.40	1.59	NS	NS	NS
Back, %	21.41	20.57	22.36	21.88	1.87	NS	NS	NS
Wings, %	14.21	14.22	15.11	14.59	0.51	**	NS	NS

S.E. –standard error; ** P<0.01; ***P<0.001.

The percentage of the breast with skin and bones were reduced in the pastured birds (P<0.01). On the other hand thighs were not affected by the rearing system. The same effect of the pasture was observed for the part of the breast and thigh muscles. Furthermore, the ratio between the red and white meat obtained from the thigh and breast muscles as presented on Figure 1 showed that the pastured chickens had reduced development of the breast muscles. The percentage of the wings was increased in the pastured birds (P<0.01), while the back was not significantly affected by the rearing strategy. In line with our observations, Batkowska *et al.* (2015) found decrease in the breast muscle proportion in extensively reared hybrid chicks, but no effect of the rearing system was found in regard to the thighs. On the other hand, the authors did not find any effect on wings while the trunk was increased in the pastured birds. Contrary to our results, Küçükiyalmaz *et al.* (2014) and Inci *et al.* (2016) did not find significant change in the percentage of breast meat respectively in broilers and quails as affected by organic rearing with pasture access, however the latter observed decrease in the drumstick ratio in the male birds on pasture, compared to the conventional rearing.



***P<0.001

Figure 1. Difference in the ratio between red and white meat in LB and BB lines reared conventionally or with pasture access

Meat chemical composition

The breast and thigh meat chemical composition (Table 3) showed that pasture access had stronger influence than the line of the birds in regard to the examined traits. Both lines reared on pasture had lower lipid content (P<0.01) in breast as well as higher moisture (P<0.001) in both breast and thigh meat when compared to the indoors reared birds. Significant interaction between the rearing system and the line, however, was observed in regard to the moisture content (P<0.05), showing that this parameter could be modified not only through the rearing strategy.

Table 3. Chemical composition of breast and thigh meat in LB and BB chickens reared conventionally or having access to pasture

Item	Conventional		Pasture access		S.E.	Significance of the factors		
	LB	BB	LB	BB		Rearing system	Line	Rearing system x Line
Breast								
Lipid, %	1.86	1.32	1.00	0.92	0.65	**	NS	NS
Protein, %	23.02	22.59	22.33	22.47	0.99	NS	NS	SN
Moisture, %	72.02	72.92	73.67	73.57	0.58	***	NS	*
Ash, %	1.14	1.19	1.01	1.05	0.03	***	**	NS
Thigh								
Lipid, %	5.40	5.85	6.01	6.16	1.44	NS	NS	NS
Protein, %	19.43	19.49	18.13	17.77	1.80	*	NS	NS
Moisture, %	72.20	71.77	72.97	73.16	0.80	***	NS	*
Ash, %	1.07	0.99	1.00	1.02	0.02	*	**	***

S.E. –standard error; ** P<0.01; ***P<0.001

In line with our results, Lin *et al.* (2014) found lower lipid content in breast in free range reared Taiwan game hens when compared to indoors reared. The authors stated that thigh lipid content was also lower in the outdoors reared birds. Küçükiyalmaz *et al.* (2012) observed no effect between organic rearing system with pasture and conventional in slow growing lines in the fat content of breast and thigh meat, however they found differences induced by the genotype in the thigh meat. On the other hand, they reported no effect of the rearing system on the moisture content. The results of the studies examining the effect of the rearing on the moisture content remain inconsistent. Dou *et al.* (2009) did not observe any difference in the moisture content in three rearing systems including also pasture access. This has been confirmed by Bartlett *et al.* (2015) and Michalczuk *et al.* (2017). Husak *et al.* (2008) reported lower moisture in organically and free range reared chickens compared with conventionally grown ones. Our results are in line with the reported by Castellini *et al.* (2002), who determined higher moisture in organically reared birds with access to pasture. Protein content did not differ between rearing systems and lines in breast, however in thigh meat significantly lower content was observed in the pastured birds, corresponding to the increased lipid content. Several studies reported increased protein content in meat of the outdoors reared birds (Fanatico *et al.*, 2007; Mikulski *et al*., 2011; Cömert *et al.*, 2016), while others did not find significant effect of the rearing systems on this trait (Wang *et al*., 2009; Sosnówska-Czajka *et al*., 2017). The ash content in breast meat was lower in the pastured birds ($P<0.001$) but significant difference was found also between the lines ($P<0.01$). In thigh meat, the values of this parameter were again found to be lower in the pastured birds ($P<0.05$), however significant dependence with the line was also observed ($P<0.001$). In contrast, with our results Küçükiyalmaz *et al*. (2012) found increased ash content in breast meat of slower growing lines reared organically with pasture access in comparison with conventionally reared, however, earlier studies (Fanatico *et al*., 2005; 2007) did not report any effect of the indoors or outdoors rearing on the ash content in the breast meat of slow-growing lines.

CONCLUSIONS

The results of this study showed that when slaughtered at the same age, the birds of both slow-growing lines reared on pasture were considerably smaller than the ones grown indoors. They had significantly reduced dressing percentage, but more developed gastrointestinal tract and higher proportion of the edible by-products. Furthermore, the proportion of breast meat was reduced while that of the wings was increased in the birds having access to pasture. In addition to the reduced contents of the breast meat, the pasture access led to significantly lower lipid content of the breast and protein in thigh, but higher moisture in both kinds of meat. Further experiments are needed, in order to find the best age for slaughter of the outdoors reared birds to compensate the effect of the pasture access on the carcass traits of the chickens and on the chemical composition of the meat.

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USING OF FUZZY LOGIC FOR DETERMINING THE APPROPRIATENESS OF PLANTING DIFFERENT AGRICULTURAL CROPS

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ABSTRACT

Selection of a particular agricultural crop for the food production is a complex problem. This is usually conditioned not only by the financial claims, but also other requirements should be taken into the account, i.e. environmental criteria, sustainability, etc. Fuzzy Logicis one of the many appropriate tools/procedures for solving such task(s).Such a procedure will be implemented within decision-making algorithm for the selection of an appropriate agricultural crop. The paper deals with the implementation of the mentioned tool/procedure for selection and ranking of the particular sort of crops, regarding different decision-making structures. Within this, there is an intention to reduce all possible biases and subjectivities to minimum by using Fuzzy Logic. This will be applied with input parameters, which are extracted and correlated with real requirements and conditions regarding actual needs of the market and farmers. Along with the offered agricultural crops and possibility of their selection, final ranking and selection of the most appropriate crop can be supported for different possible scenarios (dry or wet period of the year, accents on the financial, environmental of other criteria, available financial resources, market availability, etc.). Presented methodology will contribute to the final goal, which is systematic agricultural planting and sustainability of the food production.

Keywords: *fuzzy logic, agricultural crop, decision making, criteria, sustainability.*

INTRODUCTION

All around the world, food production is one of the importanthuman activities. Particular agricultural crops are still widely and massively planted on almost every continent. Today croplands occupy nearly 18 million km^2 (an area roughly the size of South America), which is approximately 12% of the land surface.The most abundant agricultural crops worldwide are corn, wheat, rice, rye, potatoes, sugar beets, sugar cane, pulses, soybeans, sunflower and oil palm fruit (Leff, B. et al., 2004).

In the past, a profit from production of agricultural cropswas (and still is in most of the cases, but this is changing) main factor for selection of the particular sort of the agricultural crop. Nowadays, social and especially ecological factors must be includedinto the selection analysis, i.e. crop management procedure. This implies the use of the multi-objective decision making. Paper presents asimplified methodology for the final choice between particular agricultural crops, in this case wheat and corn, which are also the most planted crops in Croatia. Wheat and corn were planned to sow on the selected location near Varaždin, Croatia. In this analysis, CALiforniaGOSym model, i.e.CALGOS model (Jones&Barnes, 2000) will be used, with modification regarding real conditions and requirements. Within this, Fuzzy Logic, i.e. Fuzzy Composite Programming will be used. Within CALGOS, such methodology was used in for three irrigation management alternatives (‘’normal/usual irrigation’’, ‘’linear move irrigation’’ and ‘’not to do irrigation’’, which were developed for each of the two soil type; sandy loam and clay loam.

MATERIALS AND METHODS

The CALGOS model was selected because this model is a modified form of a cotton crop simulation model (GOSSYM), which was presented by Baker et al., 1983 for semi-arid conditions (Marani et al., 1992), which can also be applied for the analyzed situation in Croatia. Model can provide prediction of cotton growth and development in response to variation in meteorological, soil water, and soil nitrogen conditions. Management practices in the CALGOS model (i.e. tillage, planting, irrigation, fertilizer applications) were determined from farm records and input to the crop model for the 1994 growing season(Jones & Barnes, 2000).

The aim of this paper was to provide model for selection of the optimum agriculture crop between wheat and corn for the certain soil type (a combination of sandy clay and humus). It should be noted that CALGOS model was developed for the semi arid climate and cotton crop. Despite this,CALGOS will be applied with Fuzzy Composite Programming.After this, next step in the research would be to test mentionedmethodology on real case study.

Composite programming is a normalized multi-level based methodology that deals with problems of a hierarchical nature, i.e., when certain criteria contain a number of sub-criteria. It was developed by Bardossy et al. (1985) from compromise programming. This technique, first developed by Zeleny (1973), is a mathematical programming technique that employs single level non-normalized distance based methodology to rank a discrete set of solution according to their distance from an ideal solution.

Composite programming applies Equation (1) to each sub-criterion within the same group, and then combines the compromise distance metrics of each sub-criterion to form a single composite distance metric. Then the process iterates with the successive level until final level composite distance metric is reached (one composite distance metric for each alternative)(Jones & Barnes, 2000).

$$L_j^{'} = \left[\sum_{i=1}^{n_j} (w)_{i,j} \times \left(S_j^{'}\right) p_j \right]^{1/p_j} \quad (1)$$

$L_j^{'}$ iscomposite distance for group*j* of the indicators, $S_{i,j}^{'}$ is normalized fuzzy value of the input element indicator*i* in group*j*; $(w)_{i,j}$ areweights expressing the relative importance of indicators in group *j*such that their sum is 1;$p_j$is balancing factors among indicators for group *j*, and n_j is number of indicators in group *j*. In this example*j* = 1, since there is only one group, as is shown in Table1. There are two indicators (Profitability and environment) in this group, sotherefore $n_j = n_i = 2$.

The addition of fuzzy set theory (Zadeh, 1965) to compromise programming to represent uncertainties of indicator forms fuzzy compromise programming. Similar to normalization, multilevel composite programming, fuzzy compromise programming can also be extended to normalized multi-level distance based methodology to account for uncertainties,(Jones & Barnes, 2000).

The uncertainties inherent in the indicators wereaccounted for with the use of possibilities approach. Fuzzy compromise programming is extended to a normalized multi-level distance based methodology with the use of best and worst first-level indicator values (Bogardi, 1992; Hagemeister et al., 1996), equation (2).

$$S_i^{'} = \frac{Z_i^{'} - Z\min_i}{Z\max_i - Z\min_i}, \text{ when } Z\max_i \text{ is best, or}$$

(2)

$$S_i^{'} = \frac{Z\max_i - Z_i}{Z\max - Z\min_i}, \text{ when } Z\min_i \text{ is best}$$

Where $S_i^{'}$ is normalized *i*-th fuzzy indicator; $Z_i^{'}$ is value of the *i*-th fuzzy indicator; $Z\max_i$ is maximum possible value of the *i*-th indicator; and $Z\min_i$ is minimum possible value for the *i*-th indicator.

The normalization formula presentedabove can have different form,which depend on whether the maximum is the “best” or “worst” value.It should be noted that this normalization process will result in the coordinate (1, 1) to be the ideal (best) point. Prior to examining alternatives, the decision maker (DM) must assign weights to indicate their preferences to the relative importance of indicators in the same group. The method of assigning weights to indicator is not typically defined or thoroughly documented. It usually depends on the judgement and experience of the expert group which is involved in procedure of the decision making. Most of the applications of FCP method, mentioned above, use crisp numbers to express weights according to the judgment of DM, except that of Lee et al. 1991 andLee et

al. 1992, who used the Analytic Hierarchy Process (AHP) (Maksimović et al., 2004).

The DM is also required to determine balance factors in order to evaluate alternatives using FCP. Balance factor determine the degree of compromise between indicators of the same group. Low balance factors are used for a high level of allowable compromise among indicators of the same group. Balance factor of 1 suggests that there is a perfect compromise between indicators of the group. If the level of compromise between indicators is moderate, a balance factor of 2 will be sufficient. A balance factor of 3 or higher indicates that there is minimal compromise between indicators (Jones & Barnes, 2000).

RESULTS AND DISCUSSION

Analyzed location wasarea of 5 hectares, usually planted with wheat or corn, and it is located near Varaždin, Croatia.Soil type is sandy loam soil. Considering mentioned agriculture crops, profit and required amount of the fertilizers with regards to the recommendations from CALGOS model, Table 1 presents assignment of weights and balancing factor for decision model, which were obtained by the expert group, i.e. authors of the paper during communication with the potential users of the presented methodology.

Table 1. Assignment of weights and balancing factors

Balancing factor	Group	Weights
3	Profitability	$w_1 = 0,5$
	Environment	$w_2 = 0,5$

This analysis will take into account two criteria, i.e. contributing/competing factors, which are Profitability and Environment. Both of them will be described by crisp values. Profitability is described with final profit, which is made of investment and selling price, expressed in Euros per hectare, while environment is described with amount of the fertilizer per hectare. With regards to the actual prices and data (AgroKlub,2011;AgroKlub, 2013; AgroKlub, 2017; Pioneer, 2003; Pinova, 2014; TISUP, 2017), all required input values are shown in Figure 1.

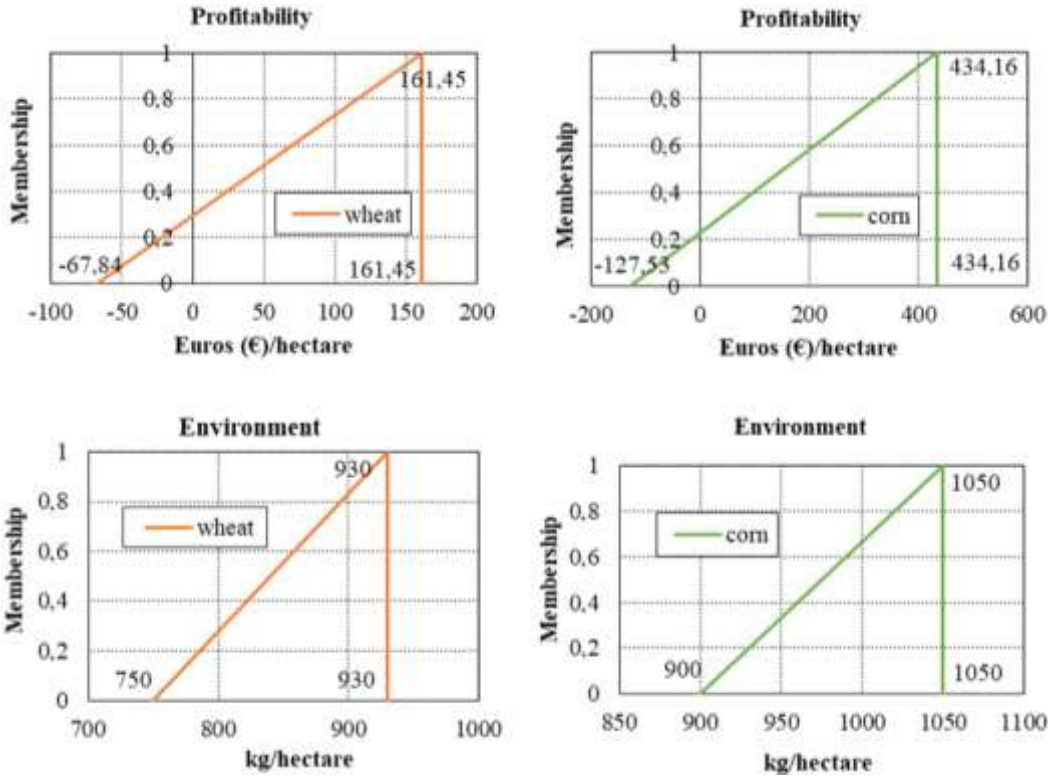

Figure 1. Description of contributors for wheat and corn

For the purpose of the calculation, computational algorithms require normalized values; therefore, ‘‘Worst’’ and ‘’Best’’ values for each contributor must be defined, table 2.

Table 2.Worst and best values for each contributor

Contributors	Worst	Best
Profitability	-127,53€/ha	434,16 €/ha
Environmental	1050 kg/ha	750 kg/ha

By using data from Figure 1 and Table 2, and by using of equations 1-2, obtained results can be seen on Figure 2. It should be noted that sensitivity analysis was also done with changes of the weights with regards to the importance of each contributor. Weightsw_1 and $w_2$were changed by ± 10 % (0.1) to see how this change affects final ranking of the variants (wheat and corn).

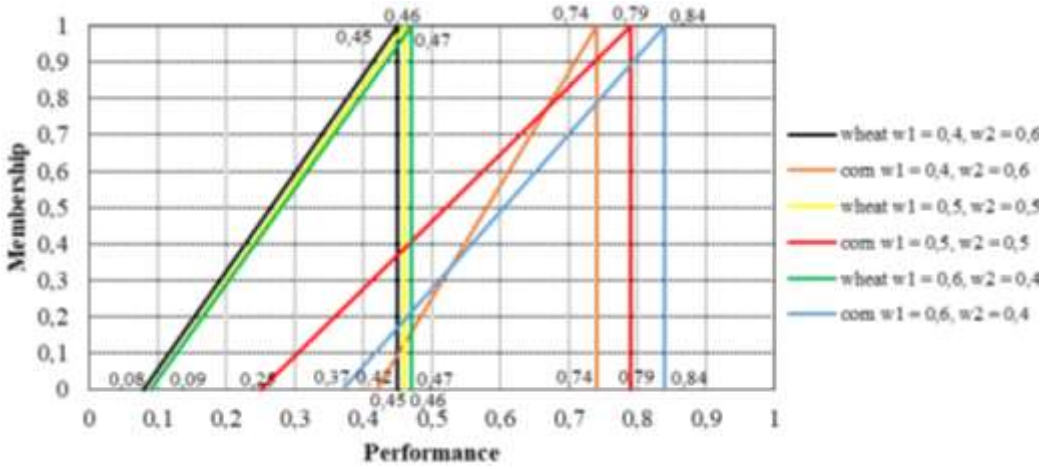

Figure 2. Fuzzy performance and rankings of the obtained variants (wheat and corn)

It can be seen that corn is better ranked crop compared with wheat, regarding all contributors and weights. Variants, which have bigger weights regarding profitability, are closest to the ideal point (1, 1). In this case, ranking i.e. ‘’defuzzyfication’’ is very obvious, due to un-ambiguity and visibilityof the shape (triangle) of each variant.

CONCLUSION

Presented methodology, although simplified, has shown that doubts of selection between particular agriculture crops can be eliminated by using Fuzzy Logic. Such procedure makes it easier to choose the optimum agriculture crop for cultivation. On first sight uncorrelated contributors (Profitability and Environment) were connected and involved into the methodology which reduces subjectivity of selection to a lesser extent, due to the preferences of the expert group.Further development of this research implies extending of the contributors, more detailed sensitivity analysis, consideration of the different soil types and extension of the analysis with regards of the other agriculture crops, which commonly growin analysed area.

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ORGANIC SUGARCANE CULTIVATION IN TAHITI

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ABSTRACT

Organic sugarcane has a strong economic potential in Tahiti. However, there is no model for small-scale organic cultivation, and the rules enforced by the applicable standards don't always respect the agroecological principles. To determine whether a small organic sugarcane farming system is profitable or not, especially in terms of productivity, control of bioagressors (weeds, rats and pests) and human resource costs, a 1ha field of sugarcane was planted in 2015, with nine different varieties, in a machineable context under European organic standards. After two years, the cultivars used showed yields from 40 up to 100 tons/ha of cane. Regarding the control of bioagressors results were the following: 1) manual removing of weeds required 4 to 6 months after planting or after the first ratoon; 2) study of rat attacks during the maturation period showed that in a dirty field, for early varieties (18° Brix at ten months of cultivation), the cane stalks can be entirely damaged, and these attacks can even occur on canes with sugar content lower than 10%. Finally, the hand labor hoeing represented around 75% of the production costs. This result demonstrates that such organic cultivation is possible even when facing pest and weed problems. In further investigations, to improve hand labor efficiency, we will first focus on weed control using small mechanized treatments; secondly, we will aim at reducing rat infestations by the use of some unique early maturated varieties to attract and treat them locally.

Keywords: *Organic cultivation, agroecology, sugarcane, French Polynesia, Tahiti.*

INTRODUCTION

Sugarcane (Poaceae family) has been developed from decades firstly to produce sugar and secondly ethanol, paper, energy and rum. It is a multiannual crop which grows under tropical climates, producing biomass during the rainy season and sugars (mainly sucrose) during the dry season (Fauconnier, 1991).In 2016 the areas

under cultivation worldwide were about 26,77 million of hectares (ha) representing1,89 billion tons with an average yield about 70 t/ha (FAO statistic division, 2017). The areas under organic sugarcane cultivation were about 44 467 ha in 2011 (Willer & Lernoud, 2013) and increased up to 91 734ha in 2015 (Willer & Lernoud, 2017) representing 0,38% of the global world amount. Based on the IFOAM (International Federation of Organic Agriculture Movements) statistics, the certified organic producers in French Polynesia grown from21 in 2012 with 2,5 ha, up to 270 in 2015 with 167 ha, showing a great interest for this type of agriculture in these islands. In the French tropical island of Tahiti, some distilleries decided in 2015 to plant some sugarcane in order to produce high valuable rums. One of them decided to do it under full organic standards. Such a farming system has a strong potential to grow due to (1) the quick evolution of all the sugarcane cultivated surfaces from 1ha in 2015 up to 25ha in 2018 (industrial data from distilleries); (2) the high interest regarding organic productions in the world and particularly in French Polynesian; (3) the prohibition of the glyphosate herbicide which will happen in the very next year following European regulations and (4) the advantages offered by the finish product : a good shelf life, a low space storage and a high selling price. However, there is no model for small-scale organic farming systems, and the rules enforced by both European and Pacific NOAB (in French: Norme Océanienne d'Agriculture Biologique) applicable standards don't always respect the agroecological principles. There is also a big gap between the organic agriculture practiced for sugar industry and the possibilities offered by the island of Tahiti in terms of topography and land availability, the machineable lands being located mostly on ferralitic soils (Jamet, 1987). Such a gap is also regulatory and technical because (1) some of organic productions can be IFOAM certified even using destructive methods such as flame burnings to remove weeds (Ascard, 1990); (2) no sugarcane farming systems, organic fertilizers or inputs are available to start a sugarcane production in Tahiti. Contrary to the agroecological principle of proximity, almost all the goods are imported by boat from abroad and far from several thousand kilometers. Fortunately, it was quite easy to find some canes in the gardens of the Polynesians to collect the cuttings. They are different by each other regarding their morphology, but all containing good amounts of sugar. In fact we found in the bibliography (Fahrasmane & Ganou-Parfait, 1997; Artschwager & Brandes, 1958) that the Otahiti cane is a very special variety of *Saccharum officinarum* which was cultivated when Bougainville arrived in 1768. It is furthermore a good commercial argument associated to organic standard production for a high value-product. On the other hand, we didn't found technical references as how to cultivate sugarcane in Tahiti or relative to fertilization and only one reference regarding bioagressors (Hammes *et al*., 1989). To identify the varieties we found old literature (Cuzent, 1860; Henry, 1928) and some interesting data regarding the areas cultivated and the sucrose yields (Toullelan, 1986) but nothing technical to help us. We then chose to be close to agroecological improvements carried out in the island of La Réunion where some organizations such as CIRAD (in French: Centre de Coopération International en Recherche

Agronomique pour le Développement) and eRcane (private company working on sugarcane development) are developing research programs around organic fundamentals. For example, Chabalier *et al*. (2012) proposed manual weed removing on a first ratoon after hand harvesting. In this study, even if herbicides are still used they also evaluated the efficiency of natural covering following an agroecological management. The first issue is actually weed development as it is the first bioagressor regarding sugarcane growth (Marnotte *et al*., 2008). This issue was also treated by considering mechanical weed control as a very effective process (Bond & Grundy, 2000). In a second time, when the sugar content increases in the stalks, the rats could be the main problem (unpublished data from Coulis M., 2015; Hood *et al*., 1970). Such information convinces us to run a local organic production based on local varieties without using any chemicals and inputs plus a technical management proven from abroad.

MATERIAL AND METHODS

Under European and NOAB organic standards, a field about1 ha was planted in a machineable context in December 2014. A plot of about 2 835 m² was delimited to evaluates even varieties (315m² each) in 2015, and nine in 2016. Eight varieties were found around the island of Tahiti, and one was located in Taha'a in the same archipelago (Society Islands). Harvesting in private gardens produced 3,125 tons in 2015. The experiment began (without irrigation) at the end of the dry season, to harvest the first canes 12 months later (December 2015) and first ratoon 24 months later (December 2016). A specific design using stripes was organized with one variety per stripe (composed of three rows) perpendicularly to a slope of about 3%. Before planting, original vegetation composed mostly of ferns was cut. Organic fertilization was then produced to correct the desaturated soil by spreading vinasse from the distillery (20t/ha), composted equine manure (5t/ha) and dolomite (2 t/ha). Minimum soil tillage was conducted (15cm deep) before creating furrows. Manual planting using "one eye" cuttings was done in paired rows with a distance between the plants of about 50cm and 1,6m (interrows), representing 20 000 cutting stalks/ha. Weed removing was conducted manually by using a tiny hand hoe in the row and using a 4WD micro-tractor of about 16 horsepower (1,1m width) with a rotative disposer for the inter-row. Yields were estimated on 3 x 100 kg of fresh full hand-harvested canes by stripe, to get a range of data in this agricultural context. These canes were crushed one time (hand feeding three rolls 1t/h crusher), and the weight of juice was measured for each sample of 100kg (giving us the crushing yield) and also for each whole stripe plot about 315m². The Brix degree was also measured for each group (5 canes: bottom, top, and middle) with a portable visual refractometer. We then deducted the production of biomass of sugarcane per stripe. Regarding rat control, we used plastic PVC pipes placed in the stripes (2 for each group) as traps containing the rodenticide (brodifacoum 0,005%). We then counted and weighed the total amount of stalks damaged just after the shootings occurred. Finally, we added the weight of sugarcane produced and the weight of stalks damaged to get the global amount of sugarcane produced

per stripe to calculate the total yield in t/ha. The cultivation calendar for agricultural operations in the years of 2015 and 2016 is shown in table 1.The global costs for such a cultivation method were then established, separating hand labor from mechanization and inputs.

Table 1. Monthly cultural operations from plantation to the first ratoon.

	jan	**feb**	**march**	**april**	**may**	**june**	**july**	**aug**	**sept**	**oct**	**nov**
2015	G+H1	G+H1	G+H1		G+H1	R	G+H1		H2+R		H2+S
2016	G+H1		G+H1			B	G+H1 R- B	B	H2+R	H2+S	

G: rotative disposer (1 people – 1 day); R: rat treatment
S: straw removing; B: Brix degree during growth
H1: hand hoeing (5 people - 5 days) before cane inter-row closure
H2: hand hoeing (5 people – 5 days) after cane inter-row closure

RESULTS AND DISCUSSION

No competition with weeds was observed at the plantation as the field was just prepared once planting the one eye cuttings. But after only 3 to 4 weeks of culture, they started to grow again. Even if one eye cuttings are more expensive, they allow a quick plant development and may give more resistance to isolated drought which can occur in the rainy season (Gonthier, 2012). Moreover, such a technique used fewer cuttings than the conventional one, which was necessary in our context. Regarding cane production after harvest, the average yields were69,0 ton/ha in 2015 and 60,9 ton/ha in 2016. The yield difference between varieties were very high from 34,7 to 111,1 ton/ha (Table 2)with excellent reliability in 2015, which was not the case in 2016 because of the massive rat attacks occurred during the early winter. We didn't maintain the field at all during three months between April and June, creating perfect conditions for rapid rat development inside the trial even if the Brix values were less than 10° at this period (light greyed out cases in Table 3). In Hawaii Hood (1970) showed that the highest populations of rats occurred in late summer and early winter, which is in accordance with our situation. At the same time, the best sugarcane yields are usually obtained in the first ratoon (Fauconnier, 1991). It was not the case for us even adding the damaged cane to the global sugarcane weight (Table 2). It is probably due to the heavy weed pressure during the first three months without any husbandry. This result showed the importance to keep the field clean in anytime.

Table 2.Production data and yields obtained from 2015 and 2016 trials. Yields in sugarcane per stripe (t/ha). Modern varieties are the 6,7 and 8.The others are noble sugarcanes.

	juice vol. / stripe (l)		Juice yield (%)		Cane weight/stripe (kg) (1)		Number of damaged stalks		Weight of damaged stalks (kg)(2)		Cane weight/ stripe (kg) (1+2)		Cane yield (t/ha)	
	2015	**2016**	**2015**	**2016**	**2015**	**2016**	**2015**	**2016**	**2015**	**2016**	**2015**	**2016**	**2015**	**2016**
1	1686	897	69%	60%	2 443	1 495		199		467	2 443	1 962	77,6	62,3
2	1012	122	68%	48%	1 488	254	66	408	165	995	1 554	1 249	49,3	39,7
3	586	120	75%	50%	781	240	313	385	781	860	1 094	1 100	34,7	34,9
4	1210	524	68%	50%	1 779	1 048		291		625	1 779	1 673	56,5	53,1
5	1050	225	70%	55%	1 500	409		382		795	1 500	1 204	47,6	38,2
6	2100	1173	60%	55%	3 500	2 133		366		790	3 500	2 923	111,1	92,8
7	2000	1360	60%	50%	3 333	2 720					3 333	2 720	105,8	86,3
8		1400		60%		2 333						2 333		74,1
9		675		60%		1 125		450		963		2 088		66,3

Table 3. Brix data for each variety between the 6th of June and 1st of August 2016. SD : Standard deviation.

	June 6th		June 27th		July 11th		Aug. 1st	
	average	**SD**	**average**	**SD**	**average**	**SD**	**average**	**SD**
1	9,54	1,72	11,30	1,75	11,71	1,48	14,52	0,90
2	14,67	2,92	14,21	1,22	15,24	2,06	15,56	0,69
3	12,47	1,12	9,69	2,02	12,71	0,68	16,05	0,37
4	9,19	1,51	9,63	2,43	9,49	0,75	13,78	0,39
5	12,00	2,53	12,51	1,87	12,65	1,27	14,59	0,99
6	14,09	1,45	15,43	3,44	14,82	1,80	14,89	0,69
7	16,37	1,82	15,39	1,69	16,63	0,96	15,91	0,29
8	10,64	3,07	10,21	1,69	11,33	2,51	15,71	0,62
9	9,37	2,62	10,29	1,95	11,42	0,93	12,58	1,84

We noticed that standard deviation decreased month after month, due to reversal amount of sugars from the bottom to the top, the sign of over-maturation (Fauconnier, 1991). Some varieties seemed to be mature around 25th of July at eight months after the first harvest (greyed out cases). In the tables 2 and 3, regarding varieties (2, 6 and 7), Brix degrees were quite high (up to 14) since June after only six months of growth. The highest yields were obtained for varieties 6 and 7 with more than 100 t/ha. This means that we probably found some modern varieties. It is very different for the variety 2 which had yield lower than 50 t/ha where rat attacks were also very high even in 2015 and 2016. This observation is of particular interest because this variety could be used as trap for rats in and around sugarcane plantations. In term of method, it is important to first observe if the ratoons are good (4 or 5 at least) before any further investigations to identify a variety with good agricultural potential. Similarly, the variety 3 only reached a Brix about 16 in August and suffered from hefty losses regarding rat attacks. This variety could also be used as trap crop for rats, with the particularity of a different attractive period. An association with service plants could be beneficial to keep some vegetation to shelter the rats but at the same time allowing the sugarcane growth (Antoir *et al*., 2016 and non published data from a seminar on service plants, CIRAD, 2018).We also observed two groups in Table 2, separated by their yields: the varieties with yields of more than 70 t/ha (6, 7, 8) and those with less (1, 2, 3, 4, 5, 9). This result was reinforced regarding Brix degree which is still very high for 6, 7 and 8 contrary to the others. We suppose that the Otahiti canes (the noble *Saccharum officinarum*) constitute the group with the lower yields and the lower Brix. We consider that the high desaturated ferralitic soil is a limiting factor for their development as opposed to the modern varieties with can produce good yields even under bad conditions of soil fertility. To separate the *Saccharum officinarum* noble canes (which could be the Otahiti ones) from the modern varieties, some samples of each variety should be examined using cytogenetic techniques and molecular biology genotyping to compare them as Schenck *et al*. (2004) did with Hawaiian varieties. However, apart from the canes supposedly to be modern cultivars, some cultivars seem to have a good potential (1 and 4) because (1) they seem to be manageable if the calendar cultivation is correctly followed (see no rat attacks in 2015);(2) their yields are above 50 t/ha with good Brix levels, and a good sucrose yield in the early period for the variety number 1 and a little late period for the variety number 4.It means the cultivation methods used allow producing and harvesting good amounts of sugarcane. To improve them investigations should be conducted with different mechanization ways using, for example, fake seedling techniques and other tools like a cover crop to reduce weed development(Bond & Grundy, 2001), human labor and finally the production costs. We could associate it with some natural covers using the residue produced after cane crushing (bagasse) thereby reducing the needs of fertilizer inputs and at the same time fighting against weed development (Quénéhervé *et al*., 2005).On the other hand, we need to get more information about the bioagressors, to adjust the future techniques. We could also propose new chemicals (or alternative ways) to

treat rat attacks (Quénéhervé *et al*., 2005). For example, they could be made locally (to avoid the increase of inputs from abroad and the use of a synthetic product), from the bark extract of *Gliricidia sepium* as a vitamin K inhibitor like described by Berkelaar (2011).

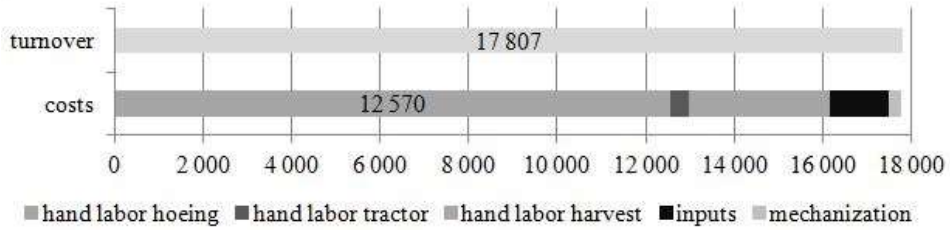

Figure 1. Turnover and production costs by category in Euros (€) / ha in 2015. Financial Balance is obtained even if hand labor hoeing represents 70,6% of the turnover.

Even if the cane closed the inter-rows between May and July (6 to 8 months after plantation or 1st ratoon), weed removing was needed until the harvest to keep the field clean to avoid rat development and make the crop easier to process. So, in Figure 1 we can easily see that the hand labor was the primary cost. It represented 90,8% of the turnover and 91,0% of the production costs. Such a result is encouraging us to continue because of the expensive selling price of the sugarcane: 500 €/t in this context of a highly valueorganic rum production.

CONCLUSIONS

We succeeded in producing a plot of organic sugarcane found locally under agroecological methods and principles. The purpose of such a farming system is to feed a highly valuable network. The first results encourage us for further investigations, improving yields and lowering costs using innovative systems.

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THE EFFECT OF COMPACTION ON WATER RETENTION IN THE VINEYARD'S ROOT ZONE

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ABSTRACT

In vineyards, frequent machinery traffic between the vine rows results in spatial and temporal changes in soil structure that affect the water retention properties in the root zone. Compaction effects on the soil water characteristic curve in the root zone were evaluated in three vineyards of different soil types (a Cl, a ClL, and a SiL with increased sand percentage). Soil cores were collected from a) the tilled soil on the vine-row and b) the compacted soil of ruts produced by machinery traffic within the inter-row distance. Sampling was carried out at two depths (0-15cm and 15-30cm) and at two time intervals, the first in spring when agricultural vehicles had accomplished 6-8 passes and the second in autumn, after ca 20 passes. The results of the first sampling in the beginning of the cultivation period revealed that compaction increased soil bulk density of the three vineyards in both depths. Drainage pores collapsed to smaller ones while plant available water and textural porosity increased. The effect of compaction was more pronounced on the surface (0-15 cm) of the more fine textured soils. In autumn, at the end of the cultivation period, it was found that the soil water retention characteristics in the vineyards root zone were not substantially further affected by machinery traffic. We concluded that machinery traffic impact on the studied properties was intense in spring when the soil in vineyards was loose from tillage before the cultivation period and had temporally increased moisture content which results in decreased strength.

Keywords: *pore size distribution, bulk density, available water, textural porosity.*

INTRODUCTION

Soil compaction in cultivated soil is mainly caused by the overuse of machinery (Saffih-Hdadi et al., 2009). In vineyards, soil tillage, chemical applications and grape harvesting lead to frequent tractor traffic. Traditional cultivation may require up to 22 passes per year, in highly mechanized viticulture (Ferrero et al., 2005). Tractors circulate in the same inter-row, which can be either between neighboring inter-rows or use all the inter-rows of the parcel (Lagacherie et al., 2006). According to Ferrero et al. (2005), the circulation of vehicles is in permanent transit corridors (ruts) located within the inter-row distance, which is usually

varying from 2.0 to 2.7m. Tractor size and slope determine the pressure exerted on the contact surface of soil. Because of the width of tractors it is often that ruts are located close to the vine row and consequently may affect soil conditions within the root zone. Soil deformation induced by mechanical stress leads to alterations in soil structure and thus modification in availability and storage of water, changes in pore continuity, tortuosity and finally in soil functions (Siczek et al., 2015). There are several soil properties that can be studied in order to determine soil compaction. For example, Saffih-Hdadi et al. (2009) suggest texture, structure and hydric state of soil. Soil compaction induced by tractor traffic increased bulk density (BD) in tilled vineyards and particularly in the portion of soil affected by the passage of tractor wheels as wheel tracks in vineyards have fixed locations (Biddoccu et al., 2016; Van Dijck and Van Asch, 2002). Moreover, significant increase in BD after traffic operations may be due to degradation of macro-aggregates into micro-aggregates, preferential loss of larger pores, and rearrangement of the micro-aggregates and primary soil particles. These changes that are more pronounced at the top soil and decrease with depth can also lead to a decrease in total porosity (TP) (Barik et al., 2014). Soil compaction reduces macro-porosity and restricts aeration and the gaseous movement system in soil–plant–air continuum. This preferential loss of larger pores is probable to change important soil hydrological functions related to water infiltration and water holding capacity and drainage. TP decreases with traffic operation and with depth. Significantly, lower TP after traffic operation is possibly due to the weight and stress effects of heavy traffic vehicles and machinery, which resulted in soil structural deterioration (Barik et al., 2014).
Soil compaction alters pore size distribution (PSD) and affects adversely soil physical fertility by impending the storage and supply of water and nutrients (Saffih-Hdadi et al., 2009). According to Głąb (2014), soil compaction influenced the soil water retention characteristics in the high matric potential range, which decreased the volume of large pores and led to an increase in volume of some fractions of smaller pores, resulting in a higher plant available water capacity. Moreover, Otalvaro et al. (2016) showed that there was a reduction of the large pores, whereas the small pores remained constant, in compacted soils. Finally, De Lima et al. (2017) suggest that reduction in soil porosity due to compaction can cause variation in pore size and in the degree of saturation, changing water retention energy. The aim of this work was to evaluate compaction effects of the circulation of machinery in two times within a cultivation period (vine blossom – May and post-harvest – October) on the soil water characteristic curve in the root zone in three vineyards of different soil texture, a Cl, a ClL and a SiL with increased sand percentage.

MATERIALS AND METHODS

Three conventionally cultivated vineyards, from the region of Amyntaion, Greece (40°41'20"N, 21°40'39"E), which varied in terms of texture, were selected to be studied. In all three vineyards similar cultivation practices were followed. The first vineyard was clayey (Cl) and planted with Syrah, the second clayey loam (ClL)

with Chardonnay the third silty loam (SiL) with an increased percentage of sand and had the cultivar Montepulcianno. Some soil properties are presented in Table 1. The three vineyards are named after their soil texture.

Table 1. Soil properties of the three vineyards.

Soil type	**Cl**		**ClL**		**SiL**	
Depth (cm)	**0-15**	**15-30**	**0-15**	**15-30**	**0-15**	**15-30**
Sand (%)[1]	18.25	22.80	25.25	24.20	41.20	34.30
Silt (%)[1]	45.95	32.70	45.95	46.15	38.60	47.30
Clay (%)[1]	31.45	40.20	26.50	28.10	12.55	12.60
Organic Matter (%)[2]	1.75	1.01	1.11	1.19	1.26	0.81
pH[3]	8.13	8.03	8.16	8.12	7.01	7.44
EC (μS/cm)[4]	747	554	733	461	403	370
$CaCO_3$ (%)[5]	6.14	9.65	42.28	41.91	0.00	0.00

[1]Pipet Method (Day, 1965), [2]Liquid Oxidation (Nelson and Sommers, 1982) [3]Soil-water suspension of 1: 2.5 (McLean, 1982) [4]Saturation Paste, [5]Electronic Limestone Calculator

The vines were planted in rows in a distance of 1.20m between them. The distance between the rows ranged from 2.30 to 2.50m and was used as the tractor's passage corridors (ruts). The first sampling took place in May 2016, when the vine was blossomed and after the vehicles had carried out 5-8 passages per rut from the beginning of the growing season. The second sampling was performed after the harvest, in October 2016 and when the vehicles had passed 15-20 times from each rut. Three undisturbed and disturbed soil samples a) uncompressed (U), between the stumps on the planting line and b) compressed (C) were obtained from each vineyard in the runway between the rows. The sampling was carried out at two depths: surface (0-15cm, depth 1) and sub-surface (15-30cm, depth 2). In all, the study comprised 24 treatments (3 vineyards*2 compression levels*2 depths*2 time intervals) with three repetitions.

The characteristic curve for soil water retention (WRC) was constructed from pairs of humidity values (h) and soil moisture (θ). Undisturbed soil cores of 4 cm in height and 5.5 cm in diameter were wetted by suction to saturation and then equilibrated in a series of suctions hj = 0, 2, 4, 10, 30, 100, 300, 600 and 1500KPa using the sand table and the high pressure ceramic plate (Klute, 1986). Total porosity was determined from the saturated water content. Pore size distribution of soil samples was determined from the WRC using the capillary rise equation for the following classes of pores with equivalent diameter >150, 150-75, 75-30, 30-10, 10-3, 3-1, 1-0.5, 0.5-0.2, 0.2-0.02 and <0.02μm and expressed as a percentage (%) of the total porosity. The same samples were used to determine BD from dry soil volume and weight. For the quantification of compression effects on the structural characteristics of pores, total porosity was divided in two major classes: the structural or inter-aggregate pores with equivalent diameter >10 μm, which are defined by the position, orientation, and shape of aggregates, and drain at matric potentials between saturation and 30 KPa; and the textural or intra-aggregate pores,

which are defined by the spatial distribution of primary soil particles and correspond to the remaining porosity when structural pores are excluded (Leij et al. 2002; Aschonitis et al., 2012). To assess the compression effects on the soil hydraulic characteristics, we evaluated the alterations of drainage pores (or air-filled porosity) with equivalent diameter >30μm and of the available water to the plants (AW) which is the water retained at matric potentials between 10 and 1500 KPa. Statistical analysis was done by ANOVA with a single factor at a significance level of 0.05.

RESULTS AND DISCUSSION

In Table 2 are shown the changes of the studied properties of the different treatments in the two time periods (blooming and post-harvest). In May, in the two fine textured vineyards (Cl and ClL) vehicle circulation increased significantly the BD, at both depths in comparison to the corresponding uncompressed samples while in the (SiL soil) compression significantly increased the bulk density only at the first depth (Table 2). Van Dijck and van Asch (2002) report that the circulation of vehicles in vineyards results in increased values of BD of both the surface soil and the subsoil due to the load exerted by the wheel. The same results between compressed and uncompressed soil samples were obtained, in October.

In the Cl vineyard, the plant available water increased after compression in most cases but the difference was significant, only in October, in both depths (Table 2). On the contrary, in the other two vineyards compaction has positively influenced available water in May and statistically significant difference is only observed between compressed and uncompressed samples in the second depth. This must be due to the collapse of bigger pores to smaller ones, after compression (Fig. 1). In all treatments, AW had a tendency to increase with depth only in the uncompressed samples. Contradictory results about the effect of compression on AW have been found by others as Barik et al. (2014), which report an increase in the volume of soil moisture after compression or Lipiec et al. (2012) who observed a decrease in available water after compaction.

Total porosity of the soil is distinguished in structural (pores> 9μm, between the aggregates) and textural (pores <9μm, within the aggregates) (Leij et al., 2002). Compression increased significantly textural porosity in all soil types at both depths and sampling times but the change was more pronounced in the surface soil (Table 2). Also, vehicle circulation decreased significantly drainage pores (>30μm) in all cases but the effect was dramatically negative in the SiL soil in May (Table 2). Moreover, the largest percentage of larger drainage pores is found in the first depth of uncompressed soil, while a statistically significant reduction of these pores was detected in second depth.

Table 2. Bulk density (BD), available water (AW) and percentage of a) drainage (>30μm) and b) textural (<10μm) pores of the first (May) and second sampling (October) for compressed (C) and uncompressed (U) soil samples of the first (1) and second (2) depth

Soil Type/ Treatment		BD (g cm^{-3})		AW (m^3 m^{-3})		Drainage Pores >30μm		Textural Pores <10μm	
		May	Oct	May	Oct	May	Oct	May	Oct
Cl	C1	1.34b A	1.24b A	0.321ab A	0.359c A	11.27a A	12.62b A	84.13b A	82.56c A
	U1	1.03a A	1.08a A	0.297a A	0.292a A	37.17b A	38.06d A	58.32a A	55.70a A
	C2	1.33b A	1.40b A	0.344bc B	0.317b A	12.70a B	3.69a A	82.12b A	91.62d A
	U2	1.15a A	1.10a A	0.374c B	0.299a A	28.36ab A	29.47c A	67.53ab A	63.86b A
ClL	C1	1.39b A	1.44b A	0.308a A	0.324a A	15.19a A	12.87a A	78.15c A	78.51d A
	U1	1.08a A	1.18a A	0.326ab A	0.329ab A	42.70c A	35.39d A	50.89a A	56.87a A
	C2	1.33b A	1.43b A	0.386c A	0.337ab A	10.11a A	21.18b B	83.05c B	71.73c A
	U2	1.18a A	1.25a A	0.347b A	0.354b A	24.05b A	28.54c A	70.06b A	64.86b A
SiL	C1	1.60b A	1.44b B	0.397ab B	0.317a A	3.92a A	21.27a B	78.83c B	58.13b A
	U1	1.21a A	1.03a A	0.375a A	0.381b A	32.88c A	38.25b A	48.34a A	46.60a A
	C2	1.46b A	1.44b A	0.428b A	0.381b A	6.16a A	12.28a A	68.05bc A	68.24c A
	U2	1.49b A	1.38b A	0.370a A	0.399b A	23.16b A	16.67a A	65.01b A	62.13bc A

*Significant differences between treatments (lowercase letters) and between the two time periods (capital letters).

No significant differences were found between the first and second sampling for the BD of all treatments in the two vineyards with fine texture. Only in the surface compressed soil of SiL vineyard there was a statistically important difference in the two time periods studied. The AW changed (increased) significantly only in the second depth of compressed treatment of the clayey soil and in the first depth of compressed treatment in the coarsest vineyard. In this treatment also changed the percentage of larger pores (>30μm), which increased post-harvest. This could be happening because the soil had reached the highest level of compaction, related to the weight of the tractors, during the cultivation period. According to Barik et al. (2014), the impact of traffic on compaction is greater under loose soil conditions.

Figure 1 presents the PSD of soil of the three vineyards between the deferent treatments for the two periods. We observe generally that the circulation of vehicles ends up in the reduction of macro-porosity and an increase of pores of smaller diameter. Liepig et al. (2012) also note the reduction of volume of larger pores, > 1–3 μm in surface and subsoil with increasing soil compaction. The

reduction of the percentage of larger pores is more pronounced in May, probably due to the looser conditions mentioned above.

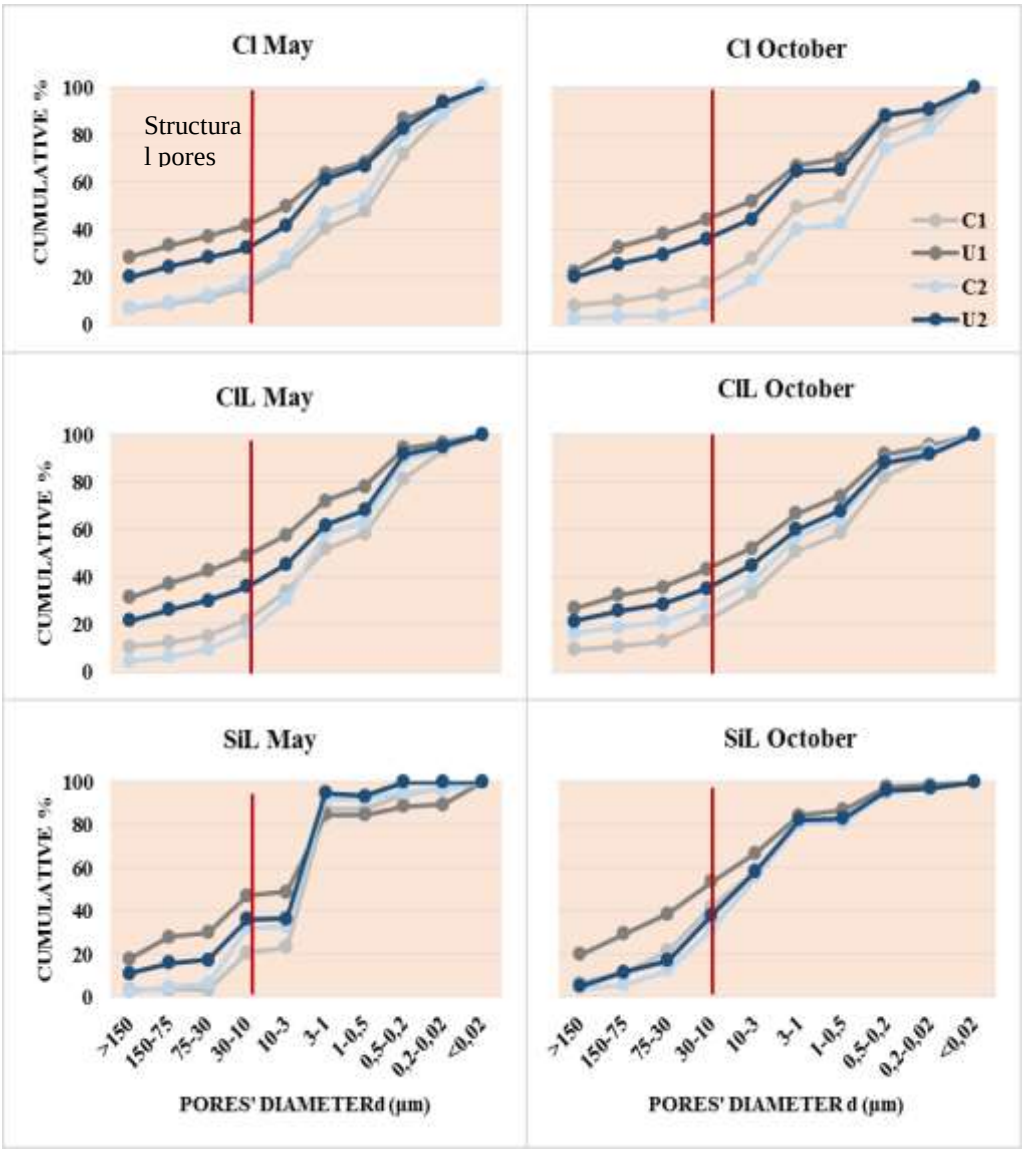

Figure 1. Pore Size Distribution (PSD) for compressed (C) and uncompressed (U) soil samples of the first (1) and second (2) depth.

CONCLUSIONS

Compaction changed the physical properties of the vineyards root zone by increasing the BD in the surface (0-15 cm) and sub-surface (15-30 cm) depth. In

addition, it shifted the pore size distribution in both periods towards the predomination of pores of smaller sizes as drainage pores collapsed to smaller ones. This fact resulted in increased textural porosity and water availability. At the end of the cultivation period, the studied properties in the vineyards root zone were not substantially further affected by machinery traffic. Soil texture affected the degree of the impact compression on BD and WRC.

From the above it is concluded, that soil compaction due to vehicle circulation in vineyards is intense in spring when the soil is loose from tillage before the beginning of the cultivation period and has temporally increased moisture content, which results in decreased strength.

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LAND COVER BASED WATERSHED HEALTH ASSESSMENT

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ABSTRACT

The adoption of appropriate managerial approaches mainly depends upon proper monitoring and consequent assessment of ecosystems health. Towards that, the watershed health monitoring has gained recognition among regulating agencies such as Environmental Protection Agency (EPA). However, its importance has not been considerably taken into account by authorities in developing countries where the outcome of such approach is essentially needed for effective and efficient management of the ever-degrading ecosystems. To this end, the present article introduces a simple and standardized approach of describing the overall watershed health situation using risk based $R_{el}R_{es}V_{ul}$ framework. Towards this, three indicators of reliability (R_{el}), resilience (R_{es}) and vulnerability (V_{ul}) have been conceptualized and calculated based on the normalized difference vegetation index (NDVI) for the Shazand Watershed, Markazi Province, Iran, as a case study. NDVI is an important and commonly used vegetation index in research on global environmental change. The primary data collected to create NDVI maps was multi-spectral satellite images of path 165 and rows of 36 and 37, with a spatial resolution of 30 m from the Landsat Satellite images for the sample year of 2014. The results of $R_{el}R_{es}V_{ul}$ analysis showed that the overall condition of the Shazand Watershed health in terms of R_{el}, R_{es} and V_{ul} was healthy, un-healthy and moderately healthy, respectively with scores of 0.82, 0.17 and 0.50 out of 1.0. The average watershed health index based on $R_{el}R_{es}V_{ul}$ framework was also obtained 0.34 varying from 0.04 to 0.46. Hence, it can be concluded that the Shazand Watershed was in relatively un-healthy state from view of vegetation cover. The maintenance and recovery of the Shazand Watershed health should be considered as fundamental step to reach the integrated watershed management objectives.

Keywords: *Health indicator, land degradation, productivity assessment, remote sensing, watershed best management.*

INTRODUCTION

Over the last decades, human pressures have unambiguously led to global environmental degradation and disruption to a degree that currently requires assessment, intervention, and remediation (Galvani et al., 2016; Liao et al., 2018). To implement remediation options, it is essential to have sound monitoring and assessment tools to know the general status of the watershed. The watershed health concept looking at a watershed as a system, instead of determining the functions of each separated part of a watershed is also implemented in other research approaches, such as soil functions, ecosystems (Keesstra et al., 2016) and the implementation of nature-based solutions to remediated degraded systems (Keesstra et al., 2017). Development of managerial tools for highlighting the valuing of ecosystem functions of watersheds is high important and valuable to manage the environment. To this end, various agencies like Environmental Protection Agency (EPA) and different researches tried to develop different watershed health monitoring tools. One of the emerging approaches developed in watershed health monitoring is the reliability, resilience and vulnerability ($R_{el}R_{es}V_{ul}$) framework initially developed by Hashimoto et al. (1982) in water resources management context. $R_{el}R_{es}V_{ul}$ was then applied to watershed health assessment with respect to water quality by Hoque et al. (2012). Consequently, $R_{el}R_{es}V_{ul}$ framework with respect to hydrological criteria was conceptualized for watershed health assessment by Hazbavi and Sadeghi (2017). In addition, Sadeghi and Hazbavi (2017) and Hazbavi et al. (2018a) applied this framework in viewpoint of drought criterion of standardized precipitation index (SPI). Recently, Hazbavi et al. (2018b) and Sadeghi et al. (2018) customized the $R_{el}R_{es}V_{ul}$ framework for different study watersheds and criteria. However, more insight investigations and minute monitoring are needed for effective and efficient management of the ever-degrading watersheds of developing countries like Iran. To this end, the present endeavor introduces a potential of a simple and standardized framework of $R_{el}R_{es}V_{ul}$ for describing the overall watershed health situation in viewpoint of land cover. The Normalized difference vegetation index (NDVI) as an important and commonly used vegetation index was therefore considered for watershed health assessment for 2014 as a sample year.

MATERIALS AND METHODS

Study Area

The Shazand Watershed (≈ 1740 km^2) is located in the southwest of Markazi Province, Iran. The watershed with 24 sub-watersheds falls within geographical coordinates from 44′42″ to 34° 12′ 13″ N and from 49° 04′ 15″ to 49° 52′ 12″ E, respectively (Figure 1). The annual mean precipitation is 430 mm and the annual mean temperature is 13.7 °C. This watershed occupies approximately 50 % highlands and hard formations, and 45 % alluvial sediments and/or sub-mountain screes. Population of the Shazand Watershed is over 102000. The Shazand Watershed has been confronted rapid urban growth and industrial development (Davudirad et al., 2016; Hazbavi et al., 2018a and b).

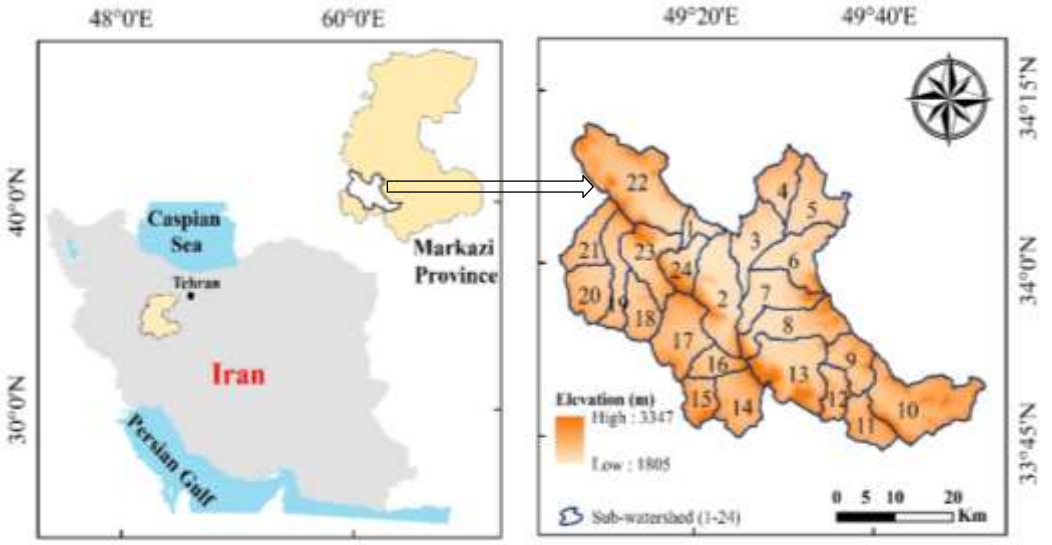

Figure 1. Location of the Shazand Watershed in Iran

Data Source

The normalized difference vegetation index (NDVI) as a representative index of land cover status was applied for the Shazand Watershed to assess the watershed health. During the recent decades, the increasing number of satellite sensors provided a great opportunity for NDVI derivation at various scales, and enabled the synergistic use of observations from multiple satellite sensors to better understand land processes. Accordingly, the data used in this study includes multi-spectral satellite images of 16-days 30-m products of path 165 and rows of 36 and 37 for year of 2014 obtained from USGS (https://earthexplorer.usgs.gov). Then the spectral reflectance measurements acquired in the near-infrared (NIR) and visible (RED) regions of the images were used based on Eq. (1) to generate NDVI in TerrSet 18.21 Software (Tucker, 1979).

$$\text{NDVI} = \frac{NIR - RED}{NIR + RED} \quad (1)$$

Conceptual Framework of Watershed Health Assessment

Three different categories of reliability, resilience and vulnerability indicators were organized to apply $R_{el}R_{es}V_{ul}$ conceptual framework. In this framework, R_{el} characterizes the frequency of failures. A failure event was defined when the watershed was failed to function within acceptable limits and was calculated from the Eq. (2):

$$R_{el} = (1 - \frac{N_r}{N}) \quad (2)$$

where N_r and N are the number of periods when the watershed is not able to meet the study criteria (failure event) and the total number of time periods in the analysis, respectively. Additionally, the R_{es} characterizes the duration of the failure events as defined in Eq. (3).

$$R_{es} = \frac{N_{fs}}{N_r} \quad (3)$$

where N_{fs} is the total number of failure sequences and N_r has the same meaning as in Eq. (2).

Furthermore, the vulnerability (V_{ul}), was defined as the average of the maximum failure occurring in each continuous failure sequence and computed through Eq. (4).

$$\text{Vulnerability } (V_{ul}) = \frac{1}{N_{fs}} \sum_{k=1}^{N} \left\{ \left[\frac{L_{obs}(k) - L_{std}(k)}{L_{std}(k)} \right] H[L_{obs}(k) - L_{std}(k)] \right\} \quad (4)$$

where N_{fs} has the same meaning as in Eq. (3), $L_{obs}(k)$ is the observed study criteria at the k^{th} time step, $L_{std}(k)$ is the corresponding compliance standard, and H[ ] is the heaviside function which ensures that only failure events were involved in calculation of V_{ul}. The heaviside function is a mathematical and discontinuous function whose value is zero for negative argument and one for positive argument (Hashimoto et al., 1982; Silva, 2010; Hoque et al., 2012). The acceptable limits or standard for NDVI status of study watershed was determined based on its mean value in the protected (exclosure) area where located in the center of the Shazand Watershed.

Three aspects of R_{el}, R_{es} and V_{ul} for NDVI criterion were accordingly computed. The aggregated $R_{el}R_{es}V_{ul}$ index was then calculated using geometric mean of standardized R_{el}, R_{es} and V_{ul} indicators (Loucks, 1997; Cude, 2001; Zhao et al., 2006; Hazbavi and Sadeghi, 2017) to provide a comprehensive characterization of a watershed ability to maintain its structure and function.

RESULTS AND DISCUSSION

The spatial distribution of NDVI in the Shazand Watershed for different months of 2014 has been presented in Table 1. Furthermore, the results of R_{el}, R_{es}, V_{ul} indicators and aggregated index based on NDVI criterion have been shown in Table 2. In addition, the spatial distribution of the land cover based watershed health index has been visualized in Figure 2.

Table 1. Spatiotemporal distribution of NDVI for different months in 2014 for the Shazand Watershed, Iran

SW	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct.	Nov	Dec
1	-0.07	-0.15	-0.13	-0.12	0.03	0.09	0.05	0.04	0.05	0.09	-0.07	-0.08
2	-0.07	-0.15	-0.14	-0.12	0.01	0.14	0.06	0.07	0.08	0.11	-0.07	-0.06
3	-0.07	-0.15	-0.14	-0.12	0.02	0.10	0.04	0.06	0.06	0.08	-0.07	-0.07
4	-0.06	-0.15	-0.13	-0.11	0.04	0.05	0.02	0.02	0.00	0.08	-0.06	-0.09
5	-0.06	-0.15	-0.13	-0.11	0.03	0.11	-0.01	0.02	0.05	0.07	-0.06	-0.09
6	-0.06	-0.15	-0.13	-0.11	0.04	0.08	0.04	0.03	0.03	0.08	-0.06	-0.09
7	-0.07	-0.16	-0.14	-0.12	0.01	0.10	-0.03	0.03	0.07	0.08	-0.07	-0.09
8	-0.07	-0.16	-0.14	-0.12	0.02	0.12	0.04	0.03	0.07	0.07	-0.07	-0.09
9	-0.07	-0.15	-0.14	-0.12	0.03	0.06	0.02	0.02	0.03	0.07	-0.07	-0.10
10	-0.06	-0.15	-0.13	-0.11	0.03	0.11	0.07	0.04	0.06	0.09	-0.06	-0.08
11	-0.08	-0.16	-0.14	-0.13	0.04	0.05	0.02	0.02	0.01	0.07	-0.08	-0.09
12	-0.06	-0.14	-0.13	-0.11	0.02	0.12	0.02	0.05	0.08	0.10	-0.06	-0.07
13	-0.04	-0.13	-0.12	-0.09	0.03	0.12	0.06	0.07	0.08	0.11	-0.04	-0.06
14	-0.08	-0.15	-0.14	-0.13	0.05	0.07	0.03	0.03	0.01	0.07	-0.08	-0.09
15	-0.06	-0.14	-0.13	-0.11	0.03	0.10	-0.02	0.03	0.06	0.08	-0.06	-0.08
16	-0.06	-0.15	-0.13	-0.11	0.03	0.09	-0.02	0.03	0.05	0.08	-0.06	-0.08
17	-0.07	-0.15	-0.13	-0.12	0.04	0.07	0.03	0.03	0.02	0.08	-0.07	-0.09
18	-0.06	-0.15	-0.13	-0.11	0.03	0.09	-0.01	0.03	0.05	0.08	-0.06	-0.08
19	-0.06	-0.15	-0.14	-0.11	0.02	0.11	0.07	0.06	0.08	0.09	-0.06	-0.07
20	-0.06	-0.14	-0.13	-0.11	0.03	0.09	0.06	0.09	0.07	0.09	-0.06	-0.07
21	-0.05	-0.14	-0.13	-0.10	0.02	0.10	0.06	0.09	0.09	0.10	-0.05	-0.06
22	-0.05	-0.13	-0.12	-0.10	0.03	0.09	0.06	0.09	0.06	0.11	-0.05	-0.05
23	-0.05	-0.14	-0.13	-0.10	0.02	0.11	0.07	0.08	0.08	0.10	-0.05	-0.06
24	-0.04	-0.13	-0.12	-0.09	0.01	0.11	0.06	0.07	0.08	0.10	-0.04	-0.06

Totally, the Shazand Watershed had no good status in viewpoint of NDVI values. The maximum, minimum, mean and standard deviation of NDVI in 2014 for the study watershed were 0.09, -0.15, -0.03 and 0.08, respectively. As seen in Table 2, R_{el} for the Shazand Watershed was almost (except sub-watersheds 7 and 16) in healthy state (= 0.89 out of one). However, the whole of the watershed except sub-watersheds 7 and 16 was in un-healthy state (= 0.11) in terms of R_{es}. The results also showed that V_{ul} varied from 0.00 to 1.00 with mean of 0.50. Despite two indicators of R_{el} and R_{es}, V_{ul} had very high variability through different sub-watersheds. The results of the aggregated land cover based $R_{el}R_{es}V_{ul}$ index revealed that two sub-watersheds of 7 and 16 which had un-healthy state in viewpoint of R_{el} and V_{ul}, were also in un-healthy state of aggregated $R_{el}R_{es}V_{ul}$ index. The healthy state of the Shazand Watershed in terms of R_{es} could not overcome the un-healthy state of other effective indicators in $R_{el}R_{es}V_{ul}$ framework. The results proved that 6, 53 and 41 % of the watershed area were categorized in un-healthy, relatively healthy and moderate healthy conditions, respectively, in viewpoint of land cover.

Table 2. Results of land cover based $R_{el}R_{es}V_{ul}$ analysis for Shazand Watershed, Iran

Indicators / Sub-watershed	R_{el}	R_{es}	V_{ul}	Land cover based watershed health index
1	0.89	0.11	0.48	0.36
2	0.89	0.11	1.00	0.46
3	0.89	0.11	0.55	0.38
4	0.89	0.11	0.11	0.22
5	0.89	0.11	0.37	0.33
6	0.89	0.11	0.28	0.30
7	0.07	0.87	0.04	0.13
8	0.89	0.11	0.56	0.38
9	0.89	0.11	0.16	0.25
10	0.89	0.11	0.63	0.40
11	0.89	0.11	0.11	0.22
12	0.89	0.11	0.66	0.40
13	0.89	0.11	0.94	0.45
14	0.89	0.11	0.22	0.28
15	0.89	0.11	0.40	0.34
16	0.07	0.87	0.00	0.04
17	0.89	0.11	0.24	0.29
18	0.89	0.11	0.31	0.31
19	0.89	0.11	0.79	0.43
20	0.89	0.11	0.69	0.41
21	0.89	0.11	0.92	0.45
22	0.89	0.11	0.77	0.42
23	0.89	0.11	0.91	0.45
24	0.89	0.11	0.80	0.43

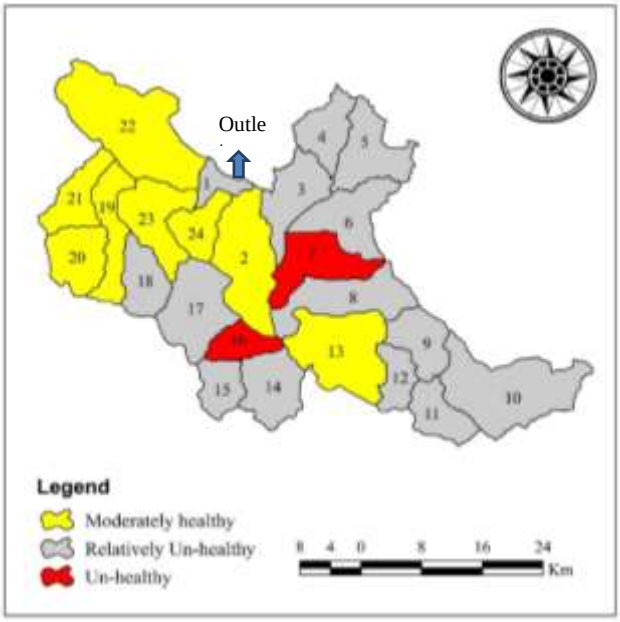

Figure 2. Distribution of land cover based watershed health index for the Shazand sub-watersheds (1-24), Iran

The vegetation cover indices such as NDVI have already been successfully applied to monitor the ecosystem state and the climatic effects (Wu et al., 2015; Damavandi et al., 2016; Sun et al., 2016; Peng et al. 2017). As Higginbottom and Symeonakis (2014) reported that an average value of NDVI < 0.1 indicating sparse biomass and influencing the soil interference, the NDVI might not be therefore considered as a good criterion. Hence, important directions for future research would be connected to the application of $R_{el}R_{es}V_{ul}$ framework with other vegetation indices to draw comprehensive conclusion on the study watershed health status.

CONCLUSIONS

Assessing watershed health based on the land cover pattern change is central for comprehensive analysis of the human-nature coupling mechanism which is seldom considered quantitatively. The current study analysed the overall watershed health situation of the Shazand Watershed, central Iran using a simple and standardized framework of $R_{el}R_{es}V_{ul}$. In contrary to R_{el} and R_{es}, V_{ul} showed large spatial variability across different sub-watersheds. In addition, the land cover watershed health index resulted from aggregation of $R_{el}R_{es}V_{ul}$ indicators were in relatively un-healthy state with value of 0.34±0.11. This method provided more accurate statistical data clarifying the local administrative responsibilities to adopt the adaptive watershed protection and restoration strategies. According to the results, it is proposed to allocate more budgets to adopt rehabilitation activities to increase the vegetation cover of the Shazand Watershed. It is highly recommended to plant native species and with low water requirement wherever industrialization and urbanization have been developed in recent years.

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SPATIOTEMPORAL ANALYSIS OF SMALL SCALE GREENHOUSE MICROCLIMATE BASED ON SMART AGRICULTURE SYSTEM

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ABSTRACT

There is a need for high cutting-edge technological ICT application in agriculture in order to embark on the current decline in agriculture labor force in Japan. However, few small-scale farmers are able or willing to risk significant capital on sensing technologies. There is a challenge in horticultural greenhouse farming to provide a well-controlled microclimate environment to meet well-developed crops with high yield and quality crop production while using fewer resources. In this study, a cost-effective simplified smart agriculture system was developed and deployed in small-scale tomato greenhouse farming in Nara, Japan. The system real-time information capability is used for monitoring crop environment for proper crop management. A spatiotemporal analysis was done to assess variations and understand the underlying microclimate conditions in the partitioned tomato greenhouse (blocks). Crop production is done all year around (An average of 2.5 times cropping cycle per one greenhouse block). Spatiotemporal analysis and statistical analysis results show well-defined micro-climate control strategies that could relatively be used in greenhouse facility management to enhance crop cultivation while using less energy resource that is relatively cost-effective. The reliability of the system data makes it efficient and consequently it could be used for accurate crop production planning, improvement in cultivation management and support in decision-making regarding cultivation activities.

Keywords: *Smart Agriculture, small scale farmer, greenhouse, spatiotemporal.*

INTRODUCTION

Shrinking agriculture workforce due to aging population and rural depopulation are alarming situations in Japanese agriculture (Nakamura, 2008). There is a concern of serious labor shortage in the near future. There is need to reinventing Japan's agriculture, this requires refined production technologies that produce high-yield, high-quality agricultural goods as well as groundbreaking, highly profitable high-

function products. Current efforts are targeting promotion of smart agriculture aiming for labor-saving and efficiency improvement using state-of-the-art robot technology and ICT (Shinichi *et al* , 2017). Smart agriculture focuses on developing production systems leading to higher-quality agricultural products.

This paper focuses on spatiotemporal analysis of small scale tomato greenhouse microclimate based on smart agriculture system. The area of this study within the controlled microclimate environment is determination of spatiotemporal conditions using wireless sensor network (WSN) collected data. According to (Nicolosi *et at*, 2017), microclimate control of greenhouse is a critical issue in agricultural practices, due to often common sudden daily variation of climatic conditions, and its potentially detrimental effect on plant growth. They further state that a greenhouse is a complex thermodynamic system where indoor temperature and relative humidity have to be closely monitored to facilitate plant growth and production. However, the daily variation of microclimate parameters i.e. temperature, relative humidity, carbon dioxide concentration, irradiation and irrigation, is not always favorable to plant growth therefore, maintaining favorable climate conditions in the greenhouse across the crop growth stages becomes necessary.

A greenhouse technology ensures a flexible and reliable solution for sustainable year-round cultivation of tomato for a relatively more cost-effective and competitive production. The farmer's tomato greenhouse used in this research, cultivate year-round tomato in six parts (blocks) within one greenhouse house. However much a greenhouse technology can provide the tomato plants with optimally-controlled microclimate growth conditions, there is still a challenge for ensuring there is well-controlled microclimate growth conditions for all the respective tomato crop growth within the greenhouse per each crop cycle. Solar radiation, temperature distribution and relative humidity are the main microclimate parameters needed to evaluate climate suitability in a greenhouse. The need to properly manage a greenhouse microclimate condition could therefore lead to significant increase in fruit quality and yields (Shamshiri *et al*, 2018). Understanding the spatiotemporal microclimate conditions would therefore lead to efficient management of facilities and ensuring a well-controlled microclimate within the greenhouse and thus reducing the excessive energy required for greenhouse heating and cooling.

MATERIALS AND METHODS

Smart Agriculture System Architecture

The smart agriculture system was deployed on already existing horticultural facilities. The system component consisted of physical components for data collection, installed WSN, database server. The software component consisted of designed database for data mining, data visualization and data analysis) as represented by the system architecture figure 1. The tomato greenhouse uses soilless cultivation technique - Nutrient film technique (NFT).

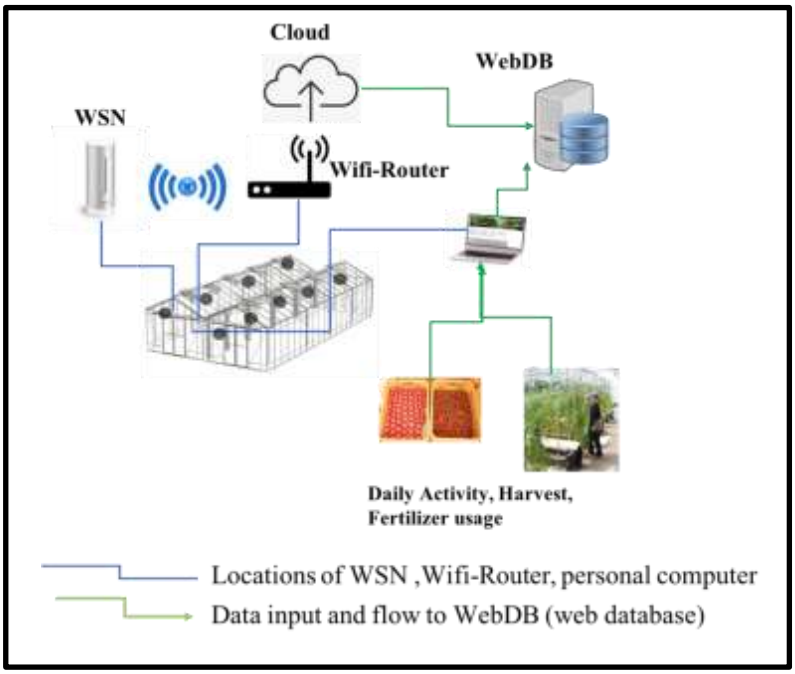

Figure 1: Smart agriculture System architecture composed of WSN for microclimate parameter data collection, Wi-Fi-Router for internet and personal computer for daily activity data entry such as harvests, fertilizer usage, crop cycles. All data is stored in the Web database (WebDB).

The smart agriculture system was installed in tomato greenhouse that is composed of six (6) blocks. WSN were distributed in the tomato greenhouse as shown in the layout figure 2. WSN measure microclimate variables air temperature, humidity, carbon dioxide concentration in the greenhouse in five minutes interval. Microclimate parameters (air temperature, carbon dioxide, humidity) between July 2017 and March 2018 were collected for analysis.

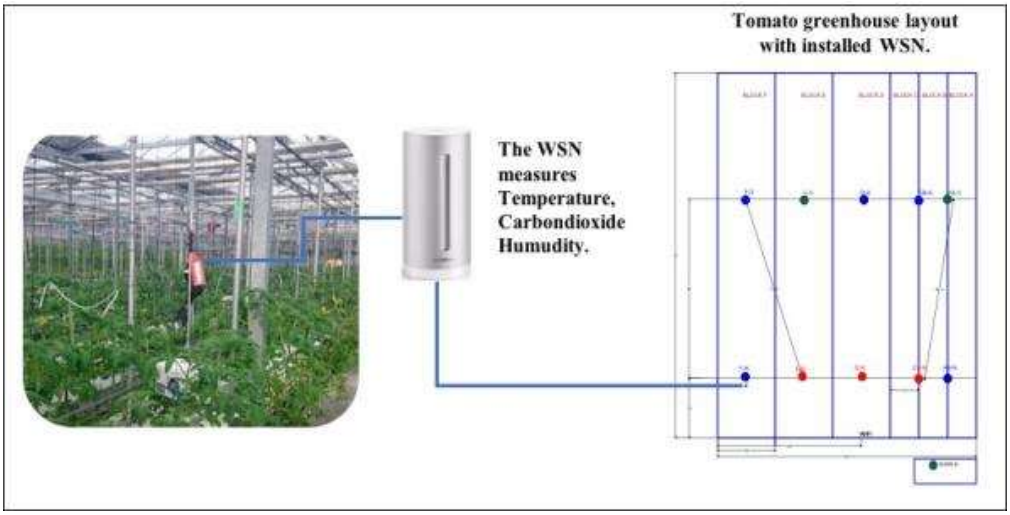

Figure 2: Tomato greenhouse layout showing the location of the installed WSN. The black, green, red points represent the location of the WSN (red points are the base module that receive data from the additional module (green points) and inside modules (blue points). The blue vertical line represent the block boundaries. y represent the length (meters) and x represent the width of the greenhouse. A total of eight (8) WSN sensors were installed in the greenhouse and one WSN in the nursery house located besides the greenhouse.

Spatiotemporal Method

The WSN measured microclimate parameters, air temperature variables used in this study, were transformed into agro-climatic index, Growing Degree Day (GDD) using degree-day method following the equation; $= \boldsymbol{\delta_i(T_{mean} - T_C)}$. Where $\delta_i = 1$ for $T_{mean} > T_C$, $\delta_i = 0$ for $T_{mean} \leq T_C$, T_{mean} is the mean daily air temperature, T_C is the threshold or base temperature (a base temperature of 10^0 was used).

To determine the spatiotemporal distribution of the microclimate environmental variables in the greenhouse, collected data of the distributed WSN data in the greenhouse were interpolated based on two-dimensional Cartesian coordinate system with the length and width of the greenhouse layout considered as x-y plane. The assumption that microclimate variables are irregularly distributed in the greenhouse, a linear method was selected, a bivariate interpolation method and smooth surface fitting for values that are given at irregularly distributed points was used (Akima, H. 1978). In this method, the x-y plane is divided into a number of triangular cells, each having projections of three data points in the plane as its vertexes. The z values are given as $z_i = z(x_i,y_i)$, where $i = 1,2, \ldots,n$. The z value in a triangle is interpolated with a bivariate fifth-degree polynomial function in x and y is applied to each triangular cell as shown by;

$$z(x,y) = \sum_{j=0}^{5} \sum_{k=0}^{5-j} q_{jk}\, x^j y^k ,$$

where z is the x, y interpolated value of the fifth-degree polynomial at each point in the x-y, q is the vertex of triangular cell with other vertices x, y at triangular points $_j$ and $_k$ of plane of the distributed WSN collected data within the greenhouse.

RESULTS AND DISCUSSION

The spatiotemporal distribution of GDD in the green house for the period between July 2017 and March 2018 showed that there were temporal variations for the monthly temperature averages. Further, spatiotemporal distribution of monthly averages of GDD and humidity microclimate variations within the greenhouse (using separate scale for each month) showed distinct variations and clearly showed how microclimate conditions within the greenhouse varied as shown in figure 3 and figure 4. The monthly averages we based on daily microclimate conditions between 6 A.M and 12 P.M. The results showed that microclimate conditions are unequally distributed.

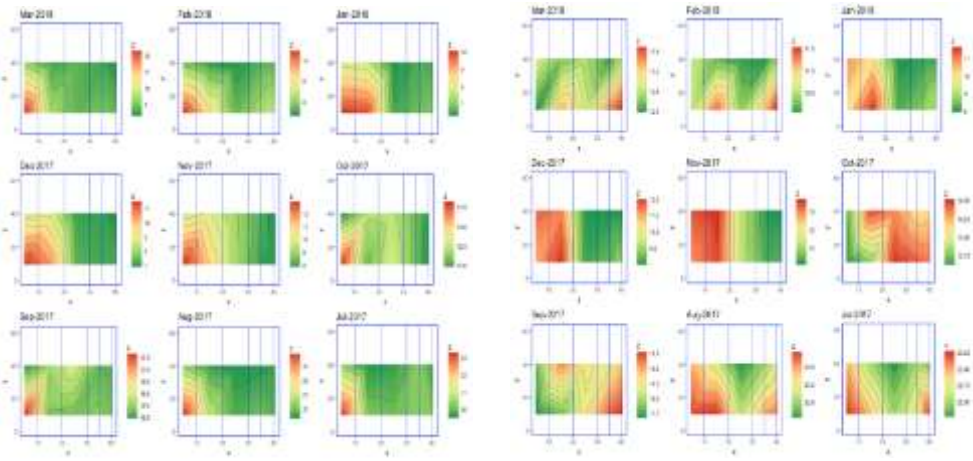

Figure 3. Spatiotemporal monthly GDD variations in tomato greenhouse. Left figure represent GDD variations between 06:00 to 12:00 in the morning and figure on right represent GDD variations between 12:00 and 18:00 in the afternoon for the period from July 2017 to March 2018.

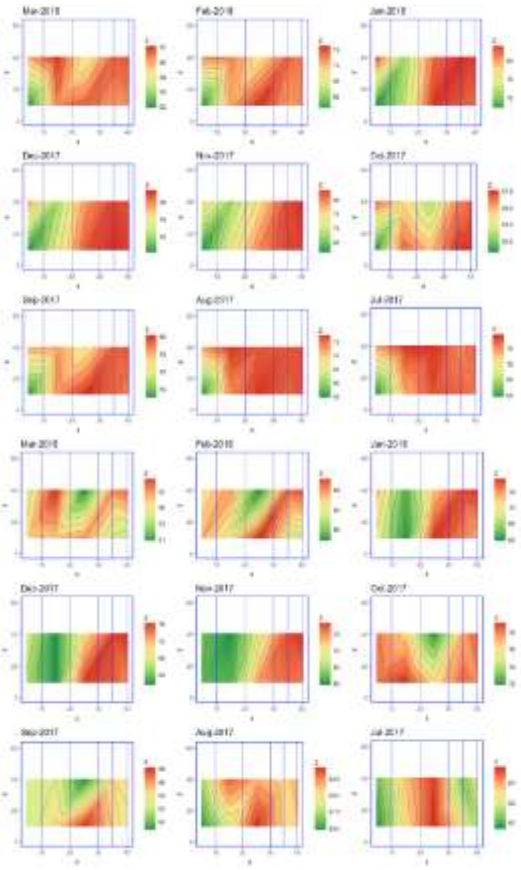

Figure 4. Spatiotemporal monthly humidity variations in tomato greenhouse. Left figure represent humidity variations between 06:00 to 12:00 in the morning and figure on right represent humidity variations between 12:00 and 18:00 in the afternoon for the period from July 2017 to March 2018.

The hourly spatiotemporal distribution was also determined. Results showed significant differences and unequal distribution during the day in the greenhouse. For microclimate conditions between 6 A.M and 12 A.M figure 5, the average GDD was between 16.2 and 24. The lowest GDD difference between the lowest and highest was 0.8 at 6 A.M. From 12 A.M to 08:00 A.M, the GDD range was between 21.5 and 24.5 with the lowest GDD difference between the lowest and highest was 0.5 at 13:00 P.M. From the spatiotemporal variation graphs, it showed distinct microclimate condition distribution and variations within the house during the day. The hotter area at specific corner in the greenhouse depicted the location of the heating facility.

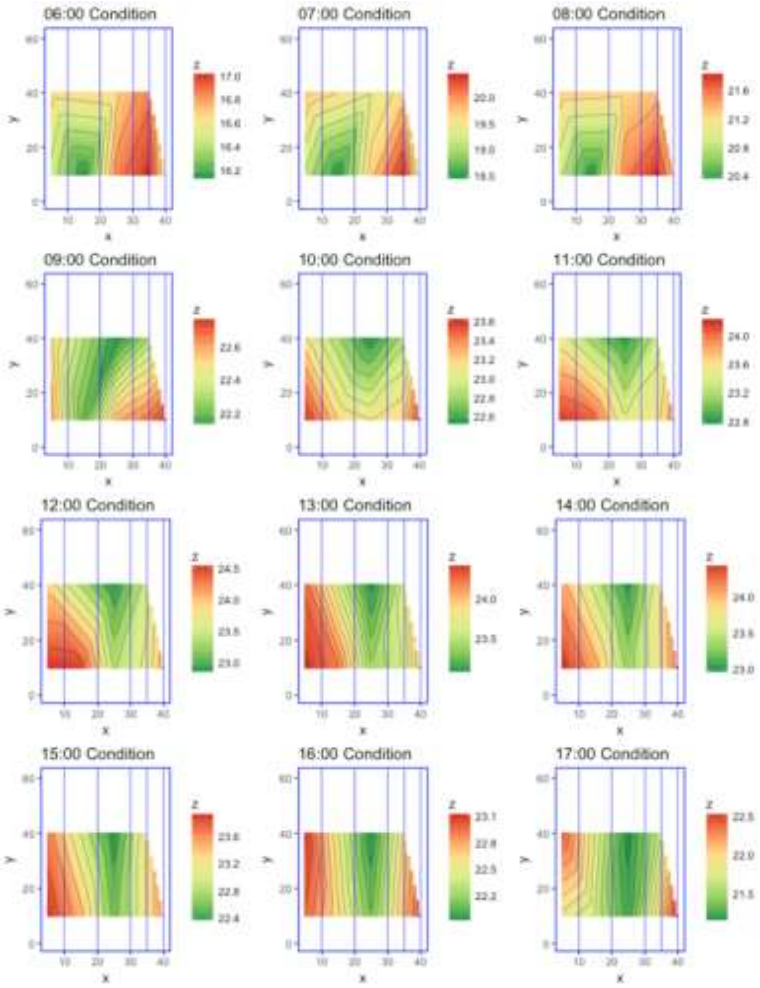

Figure 5: Daily Hourly average spatiotemporal variations between 0600 to 1800 hours for July, 2017.

CONCLUSIONS

In this study, spatiotemporal distribution of small scale greenhouse microclimate based on smart agriculture system, the spatiotemporal showed how microclimate distribution within the greenhouse varied. Further analysis of microclimate variations on an hourly basis helped understand the microclimate parameter distribution during the day within the tomato greenhouse this would be used for determining ways of ensuring recommended well-controlled and evenly distributed microclimate environment in the greenhouse by regulating heating and cooling facilities for optimum growing environment during the plant growth stage. This could also be used for improvement in cultivation management and support in decision-making regarding activities such as efficient management of facilities.

Ensuring a well-controlled microclimate within the greenhouse would thus reduce the excessive energy required for greenhouse heating and cooling.

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DEVELOPMENT OF THE DATA WAREHOUSE ARCHITECTURE FOR PROCESSING AND ANALYSIS OF THE RAW PIG PRODUCTION DATA

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ABSTRACT

Precision Livestock Farming (PLF) approach requires immense amount of data collection, aggregation and processing, using various hardware and software solutions, and is applied in many farms trying to achieve the most efficient and effective way of production. Hardware and software compatible systems capable of achieving this goal are called Farm Management Information Systems (FMIS), and are a necessity for a complete and successful implementation for Precision Agriculture (PA) branch approaches. However, most of commercially available FMIS do not only focus on crop management, but also have limited availability to small and average sized farms, in terms of price, supported language and specific features. Simpler FMIS, on the other hand, do not have necessary capabilities to fully support PLF. There are currently very small amount of high grade pig farm oriented FMIS, especially for farms with less than a hundred of sows. Therefore, there exists the need for solutions for managing farms with limited number of sows. To help address this need, authors proposed and developed architecture for unified data warehouse (DW), which was scalable and extendable cloud based data storage and processing system with support of individual data analysis. DW has capabilities to data interexchange and/or be integrated in existing FMIS throughout variety of data-in/data-out interfaces, like UIs, unmanned data supplier or consumer systems. The core of the DW is designed to provide data processing flexibility and versatility, whereas data flow within the core is organized between data vaults in a controllable and reliable way.

Keywords: *Data warehouse, information systems, pig farm management, precision livestock farming.*

INTRODUCTION

Currently the maximum efficiency and productivity of a grain or an animal farm can only be achieved by applying technological approach called Precision Agriculture (PA), as well as its branch approaches (Pierpaoli *et al.*, 2013). These

approaches allow implementation of methods that not only decrease cost of production, but also increase yields (Far and Rezaei-Moghaddam, 2018). Animal farms fall under Precision Livestock Farming (PLF) branch that is aimed to improve efficiency of production, while also increasing animal and human welfare (Banhazi *et al.*, 2012), for instance, beekeeping (Zacepins *et al.*, 2015, 2016). PLF collects and aggregates large amount of data using monitoring hardware. These data are then processed by usage of sound and/or video labelling and analysis procedures that are in most cases integrated into online automated tools capable of controlling, monitoring and modelling the behaviour of animals and their biological responses (Tullo, 2005; Nasirahmadi, Edwards and Sturm, 2017). Implementation of these automated tools, along with extended functionality, as a management software is called Farm Management Information System (FMIS).

Additionally to monitoring tools, most of commercially available FMIS provide dashboards, reporting and analysis tools, growing and feed management tools, financial management and task planning tools. Overall scope of features depends on sophistication of an offered FMIS. Most FMIS (AgWorld, FarmLogs, FarmWorks, for example) offer mainly crop management oriented tools, some (AgriWebb, for example) provide additional tools for animal management. There are also multiple highly sophisticated and feature rich FMIS like AgritecSoft and AgroSoftLtd. In the recent years development of FMIS mainly focused on online features and cloud capabilities (Welte *et al.*, 2013; Fountas *et al.*, 2015; Ampatzidis *et al.*, 2016), introducing FMIS built around different modules; therefore such FMIS, like CloudFarms, for example, are becoming highly sought after. Cloud and module based FMIS is also most appropriate system for small pig farms, as it allows to fine-tune functionality by choosing combinations of modules according to farm owner's needs. Thus not only providing required functionality, but also giving capabilities to manage overall cost of FMIS.

Despite various efforts taken by the researchers and developers to create user friendly systems for data analysis, there's still a lack of a unified, customizable and flexible systems for the pig sector. It is hard to find a universal system, which would be able to operate with different data inputs and would have flexible data processing option. Authors aim to develop an architecture for system that can be considered as decision making tool with easy and fast data entry (manual or automatic) powered by flexible and detailed reporting.

There are multiple researches aimed to differentiate requirements for pig farm FMIS (Zoranović and Novković, 2013; Husemann and Novković, 2014), including functions, modules and hardware. FMIS architecture as well has it's requirements stated in multiple researches (Murakami *et al.*, 2007; Nikkilä, Seilonen and Koskinen, 2010), that point to the need for one main centralized system, used as a hub or gateway that processes data coming from monitoring hardware. These data are then aggregated and used to create different kinds of reports, available for analysis. One of the implication of such hub is Data Warehouse (DW).

DW in its essence acts as an intermediary between data providers and data consumers (Inmon, 2002; Inmon, B, 2010), and provides customizable facilities for

data storage management, processing, analysis and output. The DW is used to help agricultural specialists run the farm more effectively. The ability to manage and effectively present the volume of data tracked in today's agriculture is the cornerstone of data warehousing. But when the data warehouse is replenished in real-time it empowers users by providing them with the most up-to-date information possible. It is possible to create capable FMIS based on DW that can be used in a combination with a Decision Support System in compliance with technical and user requirements. Authors suggest implementing Livestock Object DW as a cloud based data storage and processing unit with capabilities to combine different data sources like existing systems and available on-farm generated data. The proposed platform follows best practices in distributed and asynchronous data processing by utilizing multi-agent techniques in conjunction with real-time data warehousing approach. The aim of this paper is to describe proposed platform's architecture and functionality principles.

MATERIAL AND METHODS

The concept of proposed DW architecture (Figure 1) consists of various main components to ensure that information is stored for further analysis. Input interface provides *data-in* functionality from various sources – it can be in a form of a data file, a measurement system (configured accordingly to send data directly into DW), or third-party services etc. In most cases simple data file gives user a capability to manually upload it. Acceptable file formats are usually not heavy restricted as most systems are able to parse almost all of them. After parsing is done user should specify necessary information regarding metadata.

Data Vault modelling structure provides functionality to track history of the data flow, for instance, sender's credentials, data source and recipient. Data marts, however, are like databases (Casters, Bouman and Van Dongen, 2010) and contains a summarized information (Krneta, Jovanovic and Marjanovic, 2016) ready to be provided through a corresponding output interface.

Data are processed almost immediately after they are received at the DW. Various models are involved to ensure correct data aggregation and processing procedures that are controlled by the DW Core. DW Core is also responsible for reliable data flow management. The data are at first put into a temporary storage called Swamp and are directed to the appropriate vaults afterwards. Data flow is organized by using internal messaging service.

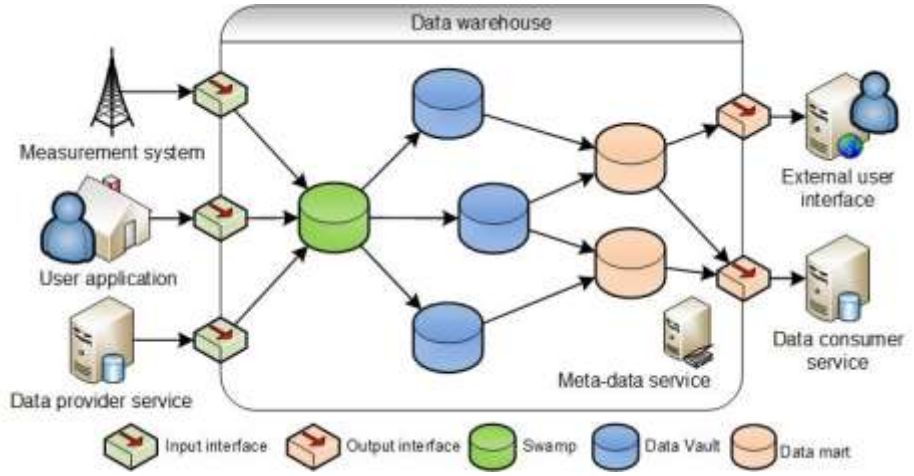

Figure 1. The concept of proposed DW architecture for data processing

Described architecture was implemented in a prototype containing several independent subsystems: DW core for handling data processing, and Web application providing graphical interface for users to submit data for processing and receive results. System was built using technologies such as Spring Boot 2.0 framework, MongoDB database to form backend and Angular 6, Bootstrap 4 framework for frontend functionality.

Results and Discussion

Overall architecture is divided into two main components – connectivity and DW Core. Connectivity corresponds to external systems' connection to DW, and includes such modules as In/Out Interfaces, which are able to use graphic user interface, and Web API that is responsible for first step authorization and verification. Each individual external system is connected to DW core using dedicated integration of Web API, where multiple Web APIs are used to connect to DW core's DataIn and DataOut services.

DW Core that provides routing and processing functionality is built using data input/output and messaging services, temporary and long term databases and processing units. DW Core's architecture contains multiple instances of Vaults, some of which are used as Marts. In proposed DW architecture Vault is a modified version of Data Vault, and differs from traditional understanding of its architecture with integrated calculation processing unit. Each Vault provides self-sufficient and logically independent transformation function, for instance, average weight per week calculation; therefore, as various raw data could be needed for a single final calculation, multiple vaults can be interconnected to build chains of data transformations resulting in flexible and extendible reporting pipeline. Vaults are responsible for processing data according to particular array of tags, such as "pigs", "weight", "week" etc.; whereas data exchange procedures are implemented in a

secure way provided by DataIn and Messaging services. DW Core includes multiple databases – one temporary storage that is used to store raw data, called Swamp, and multiple long term storages for processed data and reports, called Repository, one per each Vault.

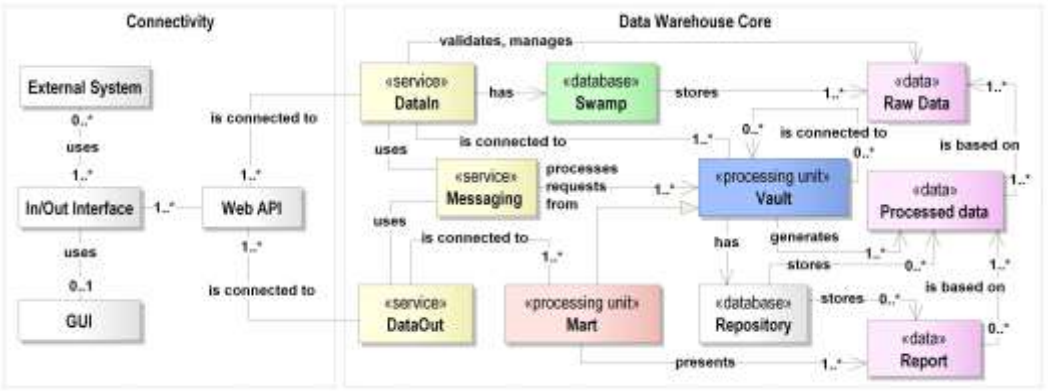

Figure 2. Proposed DW architecture and interaction between components

Proposed DW architecture was approbated using an example of manual data input and processing of these data using three vaults, one of which fulfilled the role of the Mart that was responsible for processed data aggregation and presentation in the form of report.

Data processing flow (Figure 3. Data processing flowFigure 3, [x] – step number) starts with manual data input, where user accesses one of the external systems and uploads a file, containing particular data.

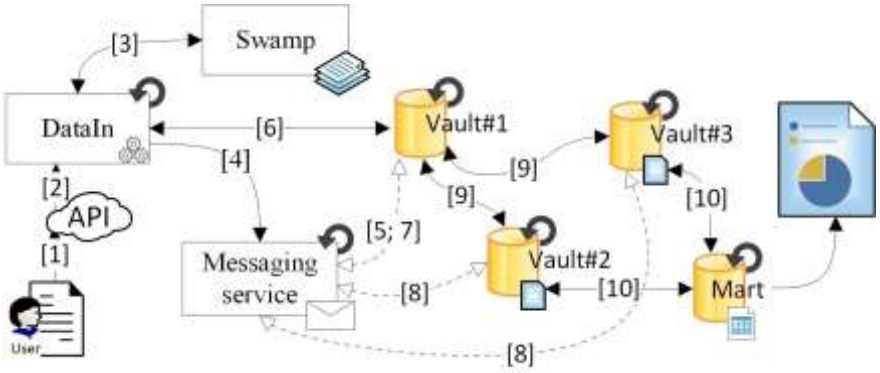

Figure 3. Data processing flow

In this instance [1], user uploaded a file containing pig weight values per day. This file is then processed by Web API that performs authorization based on user's credentials, external system's credentials and data tags included in the file. In case of successful authorization data from the file are converted to JSON format by Web API, and is forwarded [2] to DataIn service for further processing. DataIn

service performs validation and verification procedures, and upon successful acceptance transfers data [3] to the Swamp that stores all raw data. As new data are accepted, DataIn service informs [4] Messaging service about its receipt. Messaging service is responsible for informing various processing units that process these particular raw data; in this instance, Messaging service informs [5] Vault#1, that the Swamp now contains data that, based on its data tags, must be processed by this Vault. For security purposes Vaults are not connected to the Swamp; therefore Vault#1 requests these data from [6] DataIn service, that upon successful acceptance transfers requested data to Vault#1, and deletes these data from the Swamp. In proposed system, only one Vault can get and store data from the Swamp; therefore, in this instance, Vault#1 is used only as a hub that contains raw data needed by Vault#2 and Vault#3. After data receipt, Vault#1 informs [7] Messaging service about containing data with particular data tags. Corresponding Vaults are linked together using legal agreement, and receive notification [8] about data availability from Messaging service by usage of Message Queue technique. Vault#2 and Vault#3 take data [9] from Vault#1 required for different calculation functions. In this instance, Vault#2 calculates average values, and Vault#3 – maximum. Whenever there are enough processed data for reporting, Mart takes [10] these data from Vault#2 and/or Vault#3 accordingly to create a report that can be requested from DataOut service by an external system (e.g GUI) on demand and is presented to user in any acceptable format (e.g. graph, chart, table, etc). Raw data and final report resulted from processing these data, including line graphs, is shown in Figure 4.

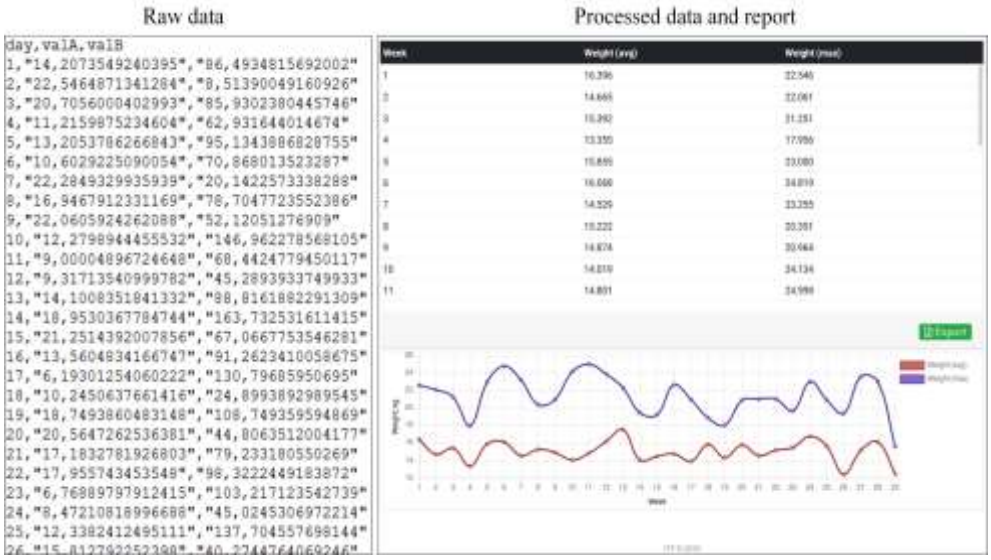

Figure 4. Results of approbated DW architecture

As seen in Figure 4, raw data, containing pig weight values per day, are processed and two calculations are made – average pig weight and maximum pig weight per

week. These calculated data are then represented in a form of two line graphs. Different output interfaces are considered, for instance, system allows exporting this report as separate pdf file, as well as accessing it from user-friendly online GUI, connected to DW through DataOut service and Out Interface. This report can also be sent to other external services and/or systems.

CONCLUSIONS

Proposed and developed architecture is universal as it allows connectivity to different multiple external systems, each of which can produce different data aimed for various livestock of PLF branches, for example, cow/pig farming, beekeeping, etc. Implemented platform provides infrastructure for data processing; however business and required calculation logic is defined by PLF industry experts and animal farm owners. Platform provides capabilities to deal with partial data, and specific cases like uploading data file with overlapping or missing data points.

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DECOMPRESSION VERSUS COMPRESSION FOUAR ANTELIAS: A GUSHING WATER RIVER

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ABSTRACT

The adopted theme in this study is the re-conquest of the Fouar Antelias river scape, the river with gushing water. After exploiting its resources in an irreversible way, today humans are facing a stage of reconciliation with the river. The materials and methods used to understand the site Fouar Antelias were firstly a speleological analysis of its springs. Then, the numeric study of the historical evolution of the bank interventions and elements using arcGIS showed the landscape lost spirit of place. Taking pictures from the same place during a year, after monthly visits, revealed the river scape dynamic temporalities. And the Cultural Value Method and Knowledge Attitude Practice method determined the social perception of the river. For the results, the suggested project on the river borders was based on the gushing water, its unique element. The adopted concept was provoking the Decompressions *vs*. Compressions explained in the speleological analysis. Deconstruction by means of Land as Art, referring to the breaking of the earth by the water Decompressions and Compressions, is the project architectural movement. The obtained plan is divided into four thematic zones from downstream till upstream. The sloped passages, the furniture emerging from the ground, the choice of plantations falling and rising and the presence of water strengthened the chosen concept. Zones of meetings, kiosks, playing area and finally a festivity zone will lead to the purpose of our linear promenade: the gushing water. Details, sections, perspectives and sketches specific to each zone supported the schematization of the concept.

Keywords: *Riverscape, Fouar Antelias, gushing water, decompression, compression.*

INTRODUCTION

The river is a stream, of different sizes, which flows into the sea. Submitted to concept of fluvial hydro system, it presents four dimensions: longitudinal, transverse, invisible and temporal (Valette, 2006). Rivers have always been a source of attraction for human; their banks were the first inhabited territory. Despite being a natural resource, water was also considered due to its seasonal

dynamism, as a constraining factor for men, a field of floods. In the face of these challenges, man built bridges to bind the banks, narrowed and curbed riverbanks to limit flooding and covered riverbeds allowing passage of roads or even constructions. The rivers were abusively exploited and thrown behind the backs of humans, considered as curses in cities. Lebanon, a Mediterranean country, has 17 perennial rivers (Khouzami et al., 1998). Its richness in streams and its diversified relief allowed the creation of many types of river landscapes: mountain, piedmont, plain and sea. The riverside civilizations occupied the borders of its rivers, the banks being places for fishing, water mills, agriculture and various occupations before becoming spaces for constructions, industries and places of parking. Our study focuses on one of the Lebanese rivers characterized by its gushing water, the Fouar Antelias. Being the smallest coastal river, its length not exceeding two kilometers, the Fouar undergoes since the beginning of XXI century an irreversible transformation in the functions of its banks. Source of irrigation for orange trees planted all over the plain of the city of Antelias in the past, this river recently constitutes a network of sanitation sewers of neighbouring cities with the invasion of urbanization. Its distinguished sources, its historic bridges and mills and its aqueduct are assets of revitalization of this neglected river landscape invaded by buildings, markets, multiple restaurants and cafes and their parking places. Given the primordial effect of a river in the life of the people, and the perpetual modifications of the banks occupations with time according to the definition of the river; given this lack of awareness of the Lebanese about rivers and their importance today, and in our case this unconcern of people towards the river and its elements in addition to the discharge of the sewers in its bed; What functions should be provided to the new banks in order to restore the lost connection of people with the Fouar river, while preserving its vestiges and rehabilitating its neglected spaces? How to illustrate its gushing water in landscaping? How to highlight its forgotten heritage elements? How to rethink the Orange plain? What concept to adopt while respecting the existing natural dynamism? How to rethink this river landscape with the increased construction of restaurants and buildings, critical elements threatening the river's potential? It is assumed that the development of a linear walk on the river may reinforce the lost link between people and the Fouar. This walk is carried out following a decompression versus compression reminding its gushing water, with zones of meeting, kiosks, playground and festivity. While taking advantage of the presence of heritage elements to create key areas, the objective of this paper is to reconnect people with rivers through riverbanks' landscaping.

MATERIAL AND METHODS

This paper is based on the site's observation during a year (June 2016 till May 2017), in order to understand it before adopting any project concept. Several methods were used to determine the physical and social/cultural aspects of the Fouar. Referring to a speleological study, the physical mechanism of water Decompression versus Compression was analysed. A cartographic comparison was

made using ArcGIS to show the evolution of the land use on the river's borders. A physicochemical test of the quality of Fouar's water was done to ensure a proper walk on the river sides. Taking photo from the same place every month helped the observation of the natural dynamism of the water, the existing riparian vegetation and the seasonal temporality of the river. The Cultural Value Method, elaborated by Stephenson was used to organize the landscape readings of the Fouar. A KAP (Knowledge Attitude Practices) survey was conducted over 50 participants, aged from 12 till 81, half of them were locals living near the river in Antelias and the other half lived at a maximum distance of 10km. Questions concerning the knowledge of the river's source and elements; their representations of the river, the necessity of the riverbanks rehabilitation project; their access to the river, if they have ever walked near the river and the activities they wish to make in the river area. The reason behind this survey was to better understand the social perception of the river and the participants' needs concerning the intervention landscape project on the riverbanks with plans, sections and perspectives taking into consideration all the obtained data.

RESULTS AND DISCUSSION

First of all, referring to the Fouar's speleological studies, the water that spurts out in Antelias comes from an underground mountain river (Hakim *et al.*, 1988). This water is subjected to a decompression, under the effect of the flow of the precipitations and the slope. It reaches an impermeable block and the decompression is transformed into an upward compression allowing the spurt of the water and giving this singularity to the Fouar. Having made an ecological inventory of the Fouar's flora, the dominant plant is the giant cane, *Arundo donax*. The mass of this plant provides an aspect of naturalness to the city, and draws the path of the river. According to a physicochemical test made on samples of water taken from the Fouar, the adoption of the idea to create a walk along the river was allowed due to the good quality of water that can be used for irrigation following the LIBNOR standards.

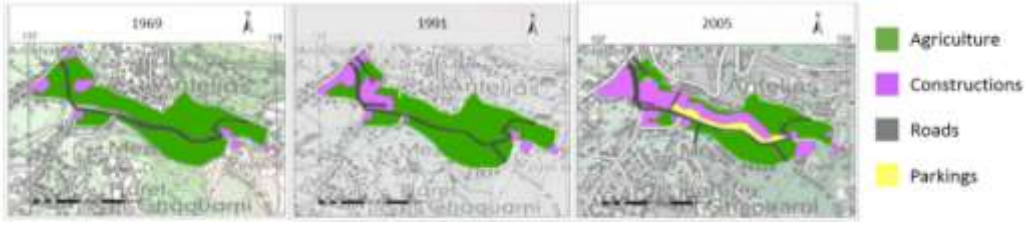


Figure 1. Evolution of the land use between 1969 and 2005

Taking into consideration the evolution of the land use next to the river, the ArcGIS study (Figure 1) showed a decrease of 44% in agricultural lands from 1696 to 2005, this decrease being the largest after the end Lebanese Civil War in 1991. This 44% is divided into 33% of buildings, 2% of new roads and 9% of parking places in 2005.

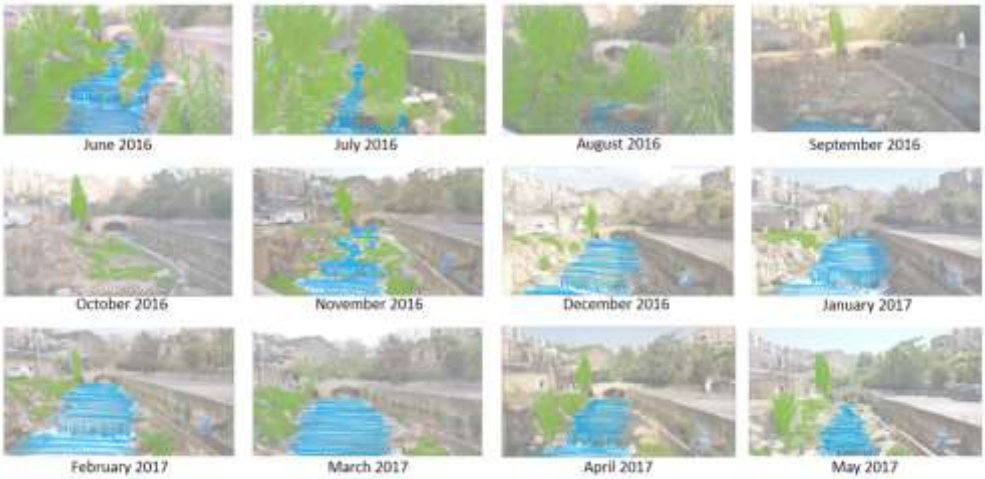


Figure 2. Kaleidoscope of the Fouar temporality during a year

Donadieu had spoken of the dynamism between nature and society in the river landscape (Donadieu *et al*., 2007). The kaleidoscope (Figure 2) expressed by taking the same photo each month, shows the natural time present in the Fouar through an interaction between water and fluvial vegetation. The level of the water decreases in the hot seasons and increases in the cold ones. Regarding vegetation, its growth is clearly visible in the first three months, before the municipality of Antelias makes a decision to 'clean up' the river, causing a break in this growing cycle over the seasons. A new cycle starts again after one month of tearing.

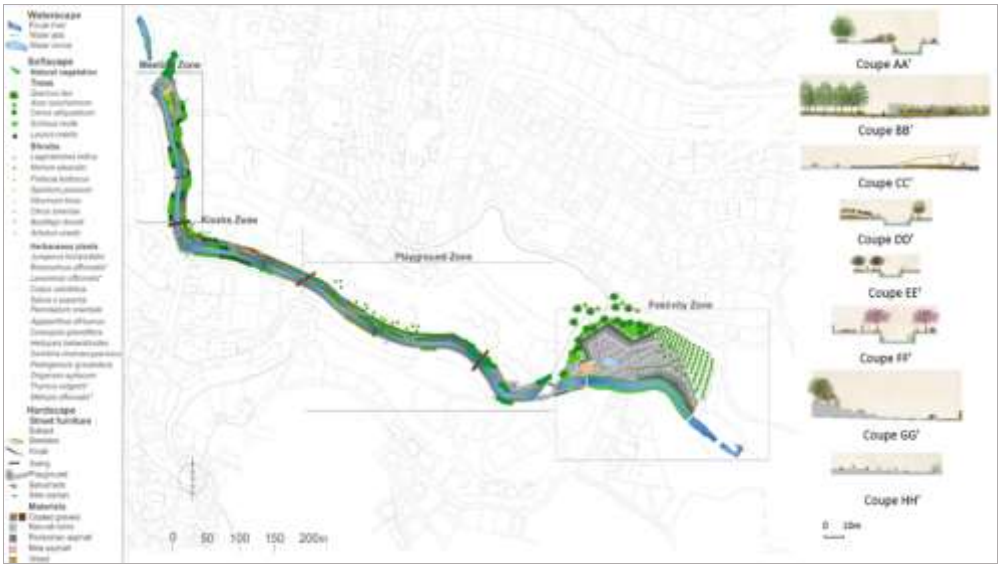
Concerning the perception of the Fouar, factors of this river were organized (Table 1) following the cultural value method (Bergstrom *et al.*, 2017) to better understand the site.

Table 1. River's perception using the Cultural Value method

Factors	**Descriptive characteristics**	**Cultural Values**		
		Forms	**Relations**	**Practice**
Flora	Continuous development, Wild, Diversity	Distinction between herbaceous strata	Endemic plant, Emblem of the river landscape	Ecological value
Water quality	Homogeneity, Color, Cleanliness	Agitated	Nourishing river	Fishing, Irrigation, Meeting place, Drinking water
River elements	Human interventions on the river	Watermills, Aqueduct, Bridges	Symbolic representation of the man-river relationship	Hydro-electric power, Water conduct; Banks link

*Source: Author's elaboration based on the site observation.

Donadieu spoke of an Object-Space that is the river and a Subject-Observer who is the man (Donadieu *et al.*, 2005). So in order to fully understand this relationship between the object and the subject through the perceptions (Luginbuhl, 2003) and sociological behaviors of people towards the Fouar and to adapt a suitable landscape project, the data of the KAP method survey were considered. 74% don't know the origin of the water' source and 76% know the existing elements like the watermills, bridges and aqueduct. The Fouar River represents for them mostly an ecology, heritage and sewers site. All the participants think that its rehabilitation is necessary. 66% have walked near the river and 78% have access. Meeting and multipurpose spaces, games and kiosks are the participants' needs concerning the project in the river area. All the inhabitants of Antelias want kiosks along the river.
So the chosen concept was: Deconstruct the land according to the Land as Art, as if it resulted from a spurt of water to provide users with the striking effect of decompression, and then that of compression. It is a linear park, divided into four areas from downstream to upstream continuously organized with furniture and vegetation specific to each. The walk is directed against the flow of water. The plot lines of the plan become more and more abruptly alternate when approaching the gushing water. The jets of water scattered every fifty meters create a chronic waving for users. The bike path is on the side of the road. The sidewalk next to it will be woven every hundred meters by written words about the Fouar and its oranges. And finally the bridges will be covered by gravel coated with a different color than those of the passages to extend their visual effect.
The depiction of Fouar's decompressions and compressions in the development of the walk on its edges will reinforce the lost link of people with this river. This walk (Map 1), which aims to reach the springing water of Fouar, is led by passages of decompressions and compressions with mineral, vegetal and aquatic elements emerging from the earth recalling this spurt. Then, the walk is divided into sequences downstream to upstream according to the expectations of local residents: meeting, kiosks, games and festivities. The mineral concept considered the 'spouting' of the furniture of the earth. The plant concept has adopted falling plants in areas of decompression and emerging in areas of compression. The stratification of these plantations referred to the strata of the riparian forest. Their mixture between deciduous and persistent recalls the seasonal dynamism, and the addition of the orange trees, reminding of the citrus plain of Antelias in the past. In addition to the existing riparian vegetation that should be kept and maintained yearly. And the water concept is manifested by the water jets dispersed in the four zones which the number increase while approaching the gushing water.



Map 1. The proposed project on the riversides

Figure 3. Meeting perspective

Figure 4. Kiosk perspective

Figure 5. Festivity perspective

In the meeting zone (Figure 3), three walks are offered: two on the banks and a seasonal one near the water. The AA' section shows the atmosphere of this meeting area, with stratified vegetation, mixed between deciduous and evergreen. The BB' section shows the passages of decompressions with falling trees, and compressions with trees and gushing plants. The plant boxes with 0.45m height serve as benches making the space versatile. In the kiosks zone (Figure 4), the dominant function is economic, kiosks selling orange based products. The CC' section shows the architectural language between the emerging bench and the kiosk. The DD' section shows the tier of dwarf orange trees, reminiscent of the Antelias citrus plain of the past, in addition to the side walk with the bike path and plant alignment. The perspective shows the general atmosphere with the bench, the kiosk and the belvedere breaking the distance between the man and the river. In the playground zone, two belvederes are present, formed by the angles of the rigid lines. A swing with corrugated roof referring to the mechanism of decompressions versus compressions offers a view towards the river. A malleable net bench is located next to the play area placed in a hollow to provide user's safety schematized in the FF' section. The vegetation becomes higher when approaching the gushing water. And the festivity zone (Figure 5) is the most agitated area, being directly connected to

the gushing water. The restored bridge is frequented by walkers in order to reach the bench. Seasonal banks will be present on the natural banks between the existing vegetation of this zone. The mill will be restored and transformed into a museum with nocturnal projections on its walls. The section GG' shows how the land is manipulated offering a view towards the aqueduct reflected in a body of water. The perspective shows the atmosphere of this area with its planted edges to reduce the noise of the road. The section HH 'shows the three passages, traced according to the natural temporality of the river.

CONCLUSION

According to the historical, physical, landscape, ecological and social study carried out on the fluvial landscape of Fouar Antelias, one reaches the various riches of this river devalued by the current riparian society. The problematic was: how to give life back to Fouar and through what functions to renew the lost link of people with him? And how to value its unique remains? The hypothesis of creating a linear walk on the river was validated.

"Increasing the ability of communities to support landscape evolutions while taking into account the singularity of places: this is the main objective of the landscape project" (Poullaouec-Gonidec. 2000). So the consideration of the singularity of Fouar, its gushing water, allowed the success of the design of the proposed project.

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ISOLATION, CHARACTERIZATION AND FORMULATION OF ANTAGONISTIC BACTERIA AGAINST FUNGAL PLANT PATHOGENS

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ABSTRACT

Concerns regarding food safety and the environment have led to reduced use of agrochemicals and the development of sustainable agriculture. In this context, biological control of fungal plant pathogens can improve global food availability, one of the three pillars of food security, by reducing crop losses, particularly for low-income farmers. Antagonistic bacteria are common soil inhabitants with potential to be developed into biofungicides for the management of fungal plant pathogens. In this study, antagonistic bacterium was isolated from the commercial compost from a Resen factory for compost and screened for its growth inhibition of fungal pathogens in laboratory tests. The zone of inhibition (mm) was recorded by measuring the distance between the edges of the growing mycelium and the antagonistic bacterium. Five replications were maintained for each isolate. Based on phenotypic characteristics, biochemical tests, and sequence analysis of 16S rRNA, the antagonistic bacterium was identified as *Paenibacillus alvei* (strain DZ-3). The bacterium suppressed the growth of all five tested fungal plant pathogens (*Fusarium oxysporum*, *Rhizoctonia solani*, *Alternaria alternata*, *Botrytis cinerea* and *Plasmopara viticola*) in *in vitro* conditions over. The survival of antagonistic bacterium in peat and talc formulations decreased time at room temperature, but the populations remained above 10^8 CFU/g during the 180-day storage period. This study suggests that this bacterium can be developed and formulated as biofungicides for minimizing the crop losses caused by fungal plant pathogens and diseases caused by them.

Keywords: *biocontrol, fungal plant pathogens, biofungicides, antagonistic bacteria.*

INTRODUCTION

As agriculture struggles to support the rapidly growing global population, plant disease reduces the production and quality of food, fibre and biofuel crops. Farmers spend billions of dollars on disease management, often without adequate technical

support, resulting in poor disease control, pollution and harmful results. In addition, plant disease can devastate natural ecosystems, compounding environmental problems caused by habitat loss and poor land management. Disease losses can mean that communities become dependent on imported foods, often replacing a balanced diet with processed foods that create further health problems.

A variety of fungi are known to cause important plant diseases, resulting in a significant lost in agricultural crops. Fungal plant diseases are considered the most important microbial agents causing serious losses in the agriculture annually (Agrios, 1988). Plant diseases caused by a variety of fungi may cause significant losses on agricultural crops. All plants are attacked by several pathogenic fungi. Each pathogenic fungi can attack one or more plants. More than 10,000 species of fungi can cause disease in plants (Agrios, 2005).

The plant diseases need to be controlled to maintain the level of yield both quantitatively and qualitatively. Farmers often rely heavily on the use of synthetic fungicides to control the plant diseases. However, the environmental problems caused by excessive use and misuse of synthetic fungicide have led to considerable changes in people's attitudes towards the use of synthetic pesticides in agriculture. Today, there is an increased awareness about the healthy food and healthy environment. In response to this, some researchers have focused their efforts on the development of plant disease control methods alternative to the use of synthetic chemicals, such as biological control using microbial antagonists. Many microbial antagonists have been reported to possess antagonistic activities against plant fungal pathogens, such as *Pseudomonas fluorescens*, *Agrobacterium radiobacter*, *Bacillus subtilis*, *B. cereus*, *B. amyloliquefaciens*, *Trichoderma virens*, *Burkholderia cepacia*, *Saccharomyces* sp., *Gliocladium* sp. (Suprapta, 2012; Pal and Garderner, 2006). Biological control of plant diseases has been considered a viable alternative method to manage plant diseases (Cook, 1993). Biocontrol is environmentally safe and in some cases the only available option to protect plants against pathogens (Cook, 1993). Biological control employs natural antagonists of pathogens to eradicate or control their population. In broad terms, biological control is the suppression of damaging activities of one organism by one or more other organisms, often referred to as natural antagonists.

In recent years, research has lead to the development of a small commercial sector which produces a number of biocontrol products. The market share of biopesticides of the total pesticide market is less than three percent. However, significant expansion is expected the upcoming decades due to the increased demand for organic food, and safer pesticides in agriculture and forestry.

Biological control agents are generally formulated as wetable powders, dusts, granules and aqueous or oil-based liquid products using different mineral, organic or inert carriers (Ardakani et al., 2009). Despite of a lot of research on biological control of plant diseases, the number of available products is limited and their market share is marginal. The market for biological control products is not only determined by agricultural aspects such as the number of diseases controlled by one biocontrol product in different crops but also by economic aspects as cost-

effective mass production, easy registration and the availability of competitive means of control including fungicides. The future development of low-chemical input sustainable agriculture and organic farming will determine the eventual role of biological control in agriculture.

The paper describes the method of isolation, characterization, biocontrol potential and formulation of antagonistic bacteria against several fungal plant pathogens.

MATERIAL AND METHODS

Origin of the bacteria- Isolation of potentially antagonistic microorganisms

Fifty grams of compost from the composting plant in Resen, Macedonia, were taken and added to 250 ml sterile distilled water in a 500 ml Erlenmeyer flask. The flask was shaken on an orbital shaker for 30 min at 27°C and serial dilutions from 10^{-1} to 10^{-6} were performed. From each dilution, 0.5 ml of sample was taken and placed on Muller Hinton agar (MHA) medium along with antimycotic cycloheximide (5 g mL^{-1}) using pour plate technique and incubated at 27°C for 1 week. After the incubation period, the plates were observed for microbial colonies which had formed a clear zone of inhibition. The colony with the greatest zone of inhibition was selected and picked up by a sterilized wire loop and sub-cultured on MHA to obtain pure bacterial colonies. The pure culture was preserved on agar slants of Muller Hinton medium for further studies.

Molecular characterization of antagonistic agents

The phenotypic properties of the selected strain wase determined using the methods described in Bergey's Manual of Determinative Bacteriology (Holt et al., 1994). The selected antimicrobial strain wase identified by sequencing of the 16S rRNA gene. First, DNA from each strain was isolated. Pure colony was grown overnight in the appropriate medium, cells were harvested by centrifugation (14000 rpm, 10 min), washed twice with 1xPBS buffer (140 mM NaCl, 2.7 mM KCl, 100 mM Na_2HPO_4, 1.8 mM KH_2PO4, pH 7.3) and kept at -20^{o}C until further processing. DNA extraction was done using PrepManUltra reagent (Applied Biosystems), following the protocol for culture broth samples. The concentration of DNA was determined spectrophotometrically. DNA working solution of 2.7 – 3.1 ng/µl was prepared by diluting the stock DNA. The sequence of the 16S ribosomal RNA gene (rDNA) of bacterial strains was determined using MicroSeq Full Gene Kit (Applied Biosystems), composed of two parts: MicroSeq® Full Gene 16S rDNA Bacterial Identification PCR Kit and MicroSeq® Full Gene 16S rDNA Bacterial Identification Sequencing Kit. Amplification of the three fragments of the 16S ribosomal RNA gene was done using 7.5 µl DNA working solution in a reaction volume of 15 µl on 2720 Thermal Cycler (Applied Biosystems). Purification of the amplified products was done using ExoSAP-IT® reagent (USB) according to the manufacturer's instructions prior to sequencing. The cycle sequencing was performed with forward and reverse primers for each amplified product according to the instructions provided by the kit with one exception: the final volume of the sequencing reactions was 10 µl. After cycle sequencing, excess

dye terminators and primers were removed from the cycle sequencing reactions by precipitation in separate tubes with 2 µl 5M Na-acetate and 50 µl ethanol. After incubation at room temperature for 30 min, the tubes were centrifuged at 14000 rpm for 30 min, the supernatant was discarded, the precipitate was dried for 5 min at room temperature and re-suspended in 20 µl of Hi-Di™ Formamide. Sequence analyses were performed on a 3500 Genetic Analyzer (Applied Biosystems).

Plant pathogens

Phytopathogenic strains (*Botrytis cinerea* FNS- FCC 23, *Fusarium oxysporum* FNS- FCC 103, *Plasmopara viticola* FNS- FCC 65, *Alternaria alternata* FNS- FCC 624, *Rhizoctonia solani* FNS- FCC 218) were supplied by the Culture Collection of the Department of Microbiology and Microbial Biotechnology, Faculty of Natural Sciences and Mathematics, Skopje, Macedonia. Fungal cultures of phytopathogenic strains were kept on Sabouraud Dextrose Agar (SDA) at 4°C.

Disc diffusion method

Disc diffusion method was used to check the antifungal properties of the isolated bacterial strain against selected fungal pathogens. Petri plates containing equal volumes of MHA and SDA (7.5+7.5 ml) medium were inoculated with a standardized bacterial isolate. A filter disc containing 20 µl of bacterial suspension was placed on a Petri plate pre-seeded with the fungal pathogen. The plates were initially kept at 4°C for 2h to allow the diffusion of the isolate, and later incubated at 28 ± 1°C. The zones of inhibition were measured after five days of incubation and the mean values were calculated. Five replications were maintained for each isolate. The zone of inhibition between pathogen and the bacterial isolate was rated as significant (+++) if the inhibition zone was >10mm wide, moderate (++) if the zone of inhibition was 2 to 10 mm wide, and poor (+) if it was <2 mm wide.

Antagonistic activity of isolated bacterium against phytopathogenic fungi

The suppressive effect and antagonistic activity of isolated bacterium against phytopathogenic fungi was demonstrated using the technique of Landa et al. (1997). Growth inhibition was expressed as the ratio of the radius of hyphal growth relative to the radius of growth on a control plate without antagonist. Values were conveniently corrected so they could be expressed in a scale from 0 (no inhibition) to 1 (maximum inhibition).

Development of talc and peat formulations of antagonistic bacteria

The formulations of selected bacterial isolate DZ-3 was prepared in talc powder and irradiated peat. The MHB broth was inoculated with a loopful of bacterium, and the flask was incubated on a rotary shaker at 150 r/min for 72 h at room temperature (24±2°C). The broth containing 8×10^8 colony-forming units (CFU)/mL, determined spectrophotometrically and by dilution plating on MHB plates, was used for the preparation of talc and peat formulation. The talc formulation was prepared with sterilized talc powder following the method

described by Vidhyasekaran and Muthamilan (1995). To 400 mL of MHB broth, the following were added under sterile conditions: 1 kg of talc powder sterilized at 105°C for 12 h, 15 g of calcium carbonate to adjust the pH to neutral, and 10 g carboxymethyl cellulose (CMC) as adhesive. The peat formulation was prepared with sterile irradiated peat. To 70 mL of MHB broth, 120 g of irradiated peat and 5 mL of bacterial culture were added under sterile conditions. The formulated products were air-dried in a laminar flow hood to a workable (15%–20%) moisture level and kept in polyethylene bags and used for the treatments immediately or as needed. The population of bacteria was around 2.5×10^8 CFU/g in both talc and peat formulations at the time of application.

Shelf life of formulated antagonistic bacteria

The shelf life of the products stored at room temperature (24±2°C) for 6 months was studied by monitoring the viability of antagonistic bacterium in peat and talc formulations by a serial dilution technique. One gram of the sample drawn from each formulation periodically at 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 150, and 180 days of storage period was mixed with 9 mL of sterile distilled water (SDW). From this, serial dilutions were made. A 1 mL aliquot of each dilution was pipetted out into sterilized Petri plates, and 15 mL of MHB was added and incubated at room temperature. The bacterial colonies were counted 3 days after plating and expressed as the number of CFU per gram of peat or talc formulation (Vidhyasekaran and Muthamilan 1995).

RESULTS AND DISCUSSION

Biological control of soil borne pathogens by introduced microorganisms has been studied over 80 years, but most of the time it has not been considered commercially feasible. However interest and research in this area increased steadily. There is a shift toward the important role of biological control in agriculture in the future. Several companies now have programs to develop biocontrol agents as commercial products. Morphological studies showed that the isolate with the greatest zone of inhibition was Gram-positive, sporulating, rod shaped bacterium. Alignment of the 16S rRNA sequences of the bacterial species revealed identity of 99% to the genus *Bacillus*. Isolate DZ-3 was identified as *Paenibacillus alvei*. Inoculated on MHA, *P. alvei* produced large, circular, rough, white-yellowish colonies with irregular margins. The spores of *P. alvei* are smooth, spherical and green in color using the Schaeffer and Fulton staining method. *Paenibacillus alvei* are Gram-positive, rod-shaped, motile, spore-forming and catalase-positive bacteria (Najafi et al., 2011). The first report of antimicrobial peptide production by these bacteria was by Anandaraj et al., 2009, who isolated a strain from fermented tomato fruit and detected two antimicrobial peptides, Paenibacillin P and Paenibacillin N. The isolated bacterial strain from compost was screened for secondary metabolites with antimicrobial activity by diffusion agar method. *Paenibacillus alvei* DZ-3 showed potential antifungal activity against all tested fungi, with the highest zones of *Alternaria alternata* FNS- FCC 624 (Table 1).

Table 1. Growth inhibition of *Paenibacillus alvei* DZ-3 on tested phytopathogenic fungi with disc diffusion method.
(Five replications were maintained for each fungus; the mean values were calculated).

phytopathogenic fungus	inhibition zone (mm)			
	Paenibacillus alvei DZ-3	+ positive control (0.5 gL^{-1} nystatine)	+ positive control (0.5 gL^{-1} cycloheximide)	- negative control (sd H_2O)
Botrytis cinerea FNS-FCC 23	11.3	5.3	7.3	0
Fusarium oxysporum FNS- FCC 103	12.1	5.4	8.7	0
Plasmopara viticola FNS- FCC 65	14.6	6.5	6.1	0
Alternaria alternata FNS- FCC 624	22.7	6.9	7.6	0
Rhizoctonia solani FNS- FCC 218	19.2	5.4	5.6	0

significant activity (+++) (inhibition zone > 10 mm)
moderate activity (++) (inhibition zone 2–10 mm)
poor activity (+) (inhibition zone <2 mm)

There are many different types of Gram positive and Gram negative bacteria (such as *Bacillus* spp. and *Pseudomonas* spp.) exhibiting antifungal activities especially toward different phytopathogenic fungi (Kobayashi et al., 2000; Gupta et al., 2001). In this group we can add our antifungal isolate *Paenibacillus alvei* DZ-3, who showed a wide range of antifungal activities toward phytopathogenic fungi.
Isolates of *Pseudomonas* were evaluated for antifungal activity against five fungal plant pathogens, i.e. *Fusarium oxysporum*, *Aspergillus niger*, *Aspergillus flavus*, *Alternaria alternata* and *Erysiphe cruciferarum* (Singh et al., 2011). All tested fungal strains showed significant reduction in terms of radial diameter after the treatment with *Pseudomonas* cultures, in comparison with the controls. Out of the five fungal pathogens studied, *Fusarium oxysporum* showed maximum extent of inhibition (% control inhibition = 51.76%) followed by *Aspergillus niger* (50.14%), and least by *Erysiphe cruciferarum* (22.27%). The antagonistic effect of *Pseudomonas* might be explained on the basis of its antifungal secondary metabolites that are capable of lysing chitin which is the most important component of fungal cell wall (Singh et al., 2011).
Biological control of plant diseases is a result of many different types of interaction among microorganisms and can occur through different mechanisms, which are generally classified as: parasitism/predation, antibiosis, competition, lytic enzymes, and induced resistance (Pal and Gardener, 2006). The most effective biocontrol active microorganisms studied appear to antagonize plant pathogen employing several modes of actions. For example, *Pseudomonas* known to produce the antibiotic 2,4-diacetylphloroglucinol (DAPG) may also induce host defenses.

Since inhibition indexes directly obtained from antagonist-phytopathogen confrontations came in different scales, they were appropriately corrected so they ranged from 0 (no pathogen inhibition) to 1 (maximum pathogen inhibition) in order to facilitate comparisons. Inhibition indexes varied widely and showed indexes from 0.12458, for *Botrytis cinerea* FNS- FCC 23 to 0.94513 for *Alternaria alternata* FNS- FCC 624 (Table 2). According to these we can conclude that the results corresponds with that from disc diffusion method.

Table 2. Inhibition indexes obtained from antagonist-phytopathogen confrontations.
(Five replications were maintained for each fungus; the mean values were calculated).

phytopathogenic fungus	*Paenibacillus alvei* DZ-3	
	inhibition index*	corrected inhibition index
Botrytis cinerea FNS- FCC 23	0.12458	0.2
Fusarium oxysporum FNS- FCC 103	0.26936	0.4
Plasmopara viticola FNS- FCC 65	0.48378	0.8
Alternaria alternata FNS- FCC 624	0.94513	1.0
Rhizoctonia solani FNS- FCC 218	0.86923	0.6

* ratio of the radius of hyphal growth relative to the radius of growth on a control plate without antagonist
0- no pathogen inhibition
1- maximum pathogen inhibition

The initial bacterial isolate *Paenibacillus alvei* DZ-3 were higher in irradiated peat than in talc powder formulation, although both products were inoculated with the same bacterial concentrations. These population densities declined over time in both formulations during the 180 days of storage at room temperature but remained above 10^8 CFU/g (Figure 1). In peat formulation, the viability of the bacterial isolate during the first 60 days of storage did not decline significantly compared with their respective initial populations of 9.4 log CFU/g at day 0 (Figure 1). The populations of antagonistic bacterium dropped to 8.72 log CFU/g after 60 days (in talk powder formulation) and to 9.1 log CFU/g (in peat formulation).

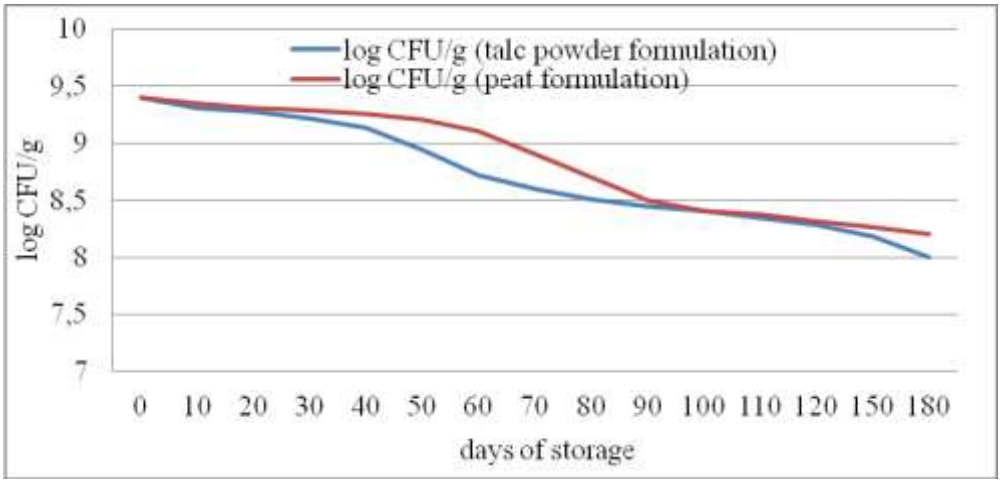

Figure 1. Population densities of antagonistic bacteria, *Paenibacillus alvei* DZ-3 in irradiated peat formulation and talc powder formulation during 180 days of storage at room temperature (24±2°C).

The main focus of the study was the performance of antifungal activity and storage stability of *Paenibacillus alvei* DZ-3, which were superior in *in vitro* conditions. In terms of the formulation for showing the storage stability it was clear that both, irradiated peat formulation and talc powder, formulation were suitable. The survival of antagonistic bacterium in peat and talc formulations decreased over time at room temperature, but the populations remained still above 10^8 CFU/g during the 180-day storage period on room temperature.

Today, the market share of biocontrol formulations is increasing and it occupies 1% of the overall pesticide sales. Montesinos (2003) and Fravel (2005) have drawn up lists of biocontrol products and strains registered by the United States Environmental Protection Agency (USEPA) and the European Protection Agency (EPA). These strains mainly belong to Bacillus and Pseudomonas bacterial genera and Aspergillus and Trichoderma fungal genera. Microbial pesticides are seen as a tool for developing a more rational pesticide use strategy and future products should have improved balance between efficiency and cost (El-Said, 2005; Rao et al., 2007; Glare et al., 2012; Khater, 2012).

Additional studies on the mechanism(s) of action of newly discovered antagonist against the tested phytopathogenic fungi are necessary to fully understand the potential beneficial role of *Paenibacillus alvei* DZ-3. In addition, field experiments are needed, particularly in regard to season long control of phytopathogenic fungi. Generally, the cost and complexity of studies for the registration of microbial pesticides is a barrier to the transfer of laboratory knowledge to the commercialization of these substances.

CONCLUSION

In order to have more effective biological control strategies in the future, it is crucial to carry out further research on certain less developed aspects of biocontrol, including development of novel formulations, understanding the impact of environmental factors on biocontrol agents, mass production of biocontrol microorganisms and the use of biotechnology and nano-technology in improvement of biocontrol mechanisms and strategies. Biocontrol of plant diseases has a bright and promising future, due to the growing demand for biocontrol products by the farmers. In addition, it is possible to use biological control as an effective strategy to manage plant diseases, increase yield, protect the environment and biological resources, and establish a sustainable agricultural system.

The study suggests that *Paenibacillus alvei* can be developed and formulated as biofungicide for minimizing crop losses and diseases caused by fungal plant pathogens .

The challenge is to develop a formulation and application method which can be implemented on a commercial scale. It must be effective, reliable, consistent, economically feasible, and with a wider spectrum. Continuous laboratory research followed by field experiments are needed to develop excellent biocontrol agents, particularly against plant fungal pathogens.

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TRACE ELEMENTS DISTRIBUTION IN HEIRLOOM PADDY PANDASAN CULTIVATED UNDER FIELD CONDITIONS OF DRY AND WET SOIL

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ABSTRACT

Trace elements phytoavailability depends on the physical and chemical properties of soil. At the Crocker range of West Coast Sabah, Malaysia, the *Pandasan* paddy variety can be cultivated as flooded rice paddies or upland rice on acidic soil. *Pandasan* paddy samples were collected in Kiulu subdistrict from traditional farmer at two different locations. Available sources of trace elements were from weathering, fertilizers and pesticides. Soil and plant samples were collected after two months of seed sowing and during harvest season which was five months old for heavy metal analysis by inductively coupled plasma optical emission spectrometry (ICP-OES). Translocation factor of arsenic from root to grain indicated this trace element was very mobile in *Pandasan* paddy cultivated at dry soil compared to wet soil followed by zinc. Although, cadmium was not detected in paddy cultivated at flooded field for both, soil and plant, cadmium was detected in soil and plant roots cultivated in dry condition. Enrichment factor results suggested that *Pandasan* plant cultivated on dry soil was only a good bioindicator for lead and zinc. *Pandasan* grain was rich with iron followed by zinc. Selected heavy metals accumulation in *Pandasan* grain cultivated in flooded field did not exceeded the permissible limit of Malaysia Food Regulation 1985. However arsenic and plumbum concentration in *Pandasan* grain harvested from dry soil exceeded the permissible limit of Malaysia Food Regulation 1985. Health risk of heavy metals toxicity can be reduced if *Pandasan* paddy is cultivated in flooded field compared to dry soil.

Keywords: *Phytoavailability, food safety, heavy metal, upland paddy, Borneo.*

INTRODUCTION

In rural agricultural land where industrial area is non-existent, heavy metal contamination can still occur through human activities such as usage of pesticides and fertilizers (Mohammed & Makame, 2015). Since the green revolution, farmers relied on man-made pesticides and fertilizers to increase plant yield. However, in long term without sustainability practices and lack of education among rural

farmers, their land productivity had declined (Mohamed *et al.,* 2016). Accumulation of high concentration of heavy metal in soil will caused phytotoxicity in plants (Alfaraas *et al.,* 2016). Subsequently threaten human health through consumption of food grown in land that contain high concentration of trace elements (Ihedioha *et al.,* 2016). Heavy metals are non-biodegradable and will continue to accumulate in agriculture soil if not decontaminated. Phytoremediation is a promising environment friendly technology using living plants to remediate contaminated soil such as heavy metals. Phytoremediation is a low cost soil remediation technique which is affordable for rural farmers to practise with the condition an easy to cultivate native plant is available as heavy metal accumulator. Rice with its high biomass is a good heavy metal accumulator and can be grown in acidic soil (Takahashi *et al.,* 2016). Heavy metal bioavailability is more readily in acidic soil. Many studies have shown that different rice species and different cultivators within different species have different characteristic ability to uptake heavy metals (Bhattacharya 2017, Duan *et al.,* 2017). However, limited data is available on indigenous paddy grown in agricultural land in Malaysia to access which cultivator or genotype is a good candidate in heavy metal soil remediation (Abdul Aziz *et al.,* 2015).

Agriculture is the main sector providing livelihood to the indigenous people living in the Crocker range. Although farmers do not only plant rice to supplement their household income, rice is the staple food (Hanafi *et al.,* 2009). Every year the diverse ethnic groups of Sabah state celebrate harvest festival and rice play an important role in cultural ceremonies. The *Pandasan* paddy variety is a traditional cultivator and not grown as commercialize paddy like the *Siam* paddy variety. As time passed and number of traditional paddy farmers decreased, *Pandasan* paddy will be less cultivated in the future as food resource. In favor of biodiversity conservation initiatives, Translocation Factor (TF) and Enrichment Factor (EF) of selected heavy metals distribution in different parts of *Pandasan* plant were analyzed to evaluate whether there are other prospect for *Pandasan* paddy not only as food source but other functions. The *Pandasan* variety is very well adapted with the climate and terrain features of the Crocker Range where it can be grown on wet and dry soil. Therefore, trace element uptake characteristic of *Pandasan* paddy from both growing conditions on wet and dry soil were assessed to identify whether *Pandasan* paddy variety can be a good specific heavy metal accumulator in ensuring sustainable agriculture in Crocker range.

MATERIAL AND METHODS

Soil and paddy plants samples were collected from sub-district of Kiulu which is located within the Crocker Range of West Coast Sabah (Malaysia) at two different villages from the same traditional paddy farmer. A minimum of random five clumps of whole paddy plants were uprooted together with soil were obtained from each location where paddy cultivated from flood plain were from the village of Kampung Poturidong Lama (6˚3' 2" N, 116˚17' 43" E), and paddy cultivated on hilly slopes depended only on rain as water resource were from Kampung Mantaranau

(6˚3' 0" N, 116˚25' 58" E). Paddy plants were harvested two times during paddy life cycle which at growing phase two months old (October 2016) and when rice grain had matured about five months old (January 2017). The indigenous paddy plant variety that were collected for this study is known as '*Pandasan*' (Accession Number IRGC 13091) by the local people which might be named according to the original location where the cultivator were grown in Pandasan area in Kota Belud district within the Crocker Range as well.

Soil collected near roots of paddy plant were air-dried. Dried soil samples were grinded with mortar and pestle. Then soil were sieved through 63µm size mesh before 1 gram of each homogenized soil samples were fully digested with aqua regia solution HNO_3:HCl (1:3) heated at 70˚C. Paddy plants were separated into three parts of roots, leaves and grains which were dried at 60˚C in oven and later ground into a fine powder. Homogenized plant samples of 1 gram were digested with 20ml HNO_3 for overnight. Then samples were heated at 120˚C in the oven for four hours. Samples were filtered with 0.45µm pore size membrane filter paper and diluted when cooled. The content of trace elements in soil and paddy plants were determined by ICP-OES (Perkin Elmer Optima 5300DV).

RESULTS AND DISCUSSION

Borneo Island is one of the oldest rainforest in the world created after exposed parent materials had formed soil as the ultimate product of continuous weathering. Soil is a medium which provide nutrients for plants to grow and produce food for consumers as they are producers in the food web chain. According to United States Department of Agriculture (USDA) soil taxonomy, a big part of terrestrial area in Borneo Island can be generally categorized as "ultisols". Characteristic of ultisols soil can be seen in the northern portion of the island of Borneo, where the Crocker range is located with visible soil profile sections of red clay soils on agricultural land which is typically acidic (Soehady Erfen *et al.,* 2016). Acidic soil can naturally increase the mobility and phytoavailability of heavy metals in soil (Abdul Aziz *et al.,* 2015). Zinc in Crocker Formation clay soil have high mobility followed by Cu, Ni, Cr, and Pb (Musta *et al.,* 2003). In the same manner, Zn availability at both wet and dry soils of this study indicates Zn mobility was also higher compared to other detected trace elements except for Fe (Table 1). Fe is more bioavailable in Malaysia agriculture areas compared to Zn (Abdul Aziz *et al.,* 2015, Khairiah *et al.,* 2013, Hanafi *et al.,* 2009). Moreover red soil is rich with iron oxides (Khairiah *et al.,* 2012). Although Fe is more bioavailable in soil compared to other trace elements, Fe appeared to be more bounded in the plant roots compared to the other parts of the *Pandasan* paddy plant (Figure 1). Zn translocation efficiency in *Pandasan* plant from root to other upper part of *Pandasan* plant is higher than Fe (Figure 2). Pandasan grain is rich in with iron and zinc notably when cultivated at dry soil (Table 1). Nevertheless, translocation of arsenic from root to grain is more efficient compared to zinc when *Pandasan* paddy were cultivated at dry soil (Figure 2).

Table 3. Mean concentration of trace elements in soil collected at different point of paddy life phase and in grain with comparison of statutory limit in food product (mg/kg) according to Malaysia Food Regulation 1985 (MFR 1985).

	Wetland			Dryland			MFR 1985
	Soil	Soil	Brown Rice	Soil	Soil	Brown Rice	
	2 months	5 months		2 months	5 months		
As	n.d.	0.34±0.00	0.12±0.00	0.24±0.09	4.38±0.06	3.38±0.6	1
Cd	n.d.	n.d.	n.d.	0.15±0.01	0.85±0.03	n.d.	1
Cr	1.03±0.00	2.75±0.03	0.11±0.00	1.16±0.01	5.87±0.09	2.10±0.03	N.A.
Cu	0.64±0.00	0.93±0.01	0.05±0.00	1.72±0.01	1.47±0.01	0.71±0.05	30
Fe	2224.09±0.62	1719.03±16.41	8.05±0.01	1242.21±5.30	1174.44±3.00	77.36±1.08	N.A.
Pb	0.46±0.00	0.98±0.02	n.d.	1.29±0.16	0.96±0.01	4.85±0.06	2
Zn	5.01±0.00	6.68±0.08	5.25±0.06	4.17±0.03	3.83±0.02	35.13±0.58	100

Notes: MFR 1985-Malaysia Food Regulation 1985, n.d.-Not detected, N.A.- Not available

During the early few months of *Pandasan* paddy growing season, water was plenty and As was not traced in the soil. However, after the same field dried up during paddy harvest season, As was traced in the soil (Table 1). In a relatively short period of time, As managed to translocate to the rice grain when soil is not waterlogged indicates amount of water in soil had an impact on phytoavailability of As. High concentration of As was detected in the rice grain of *Pandasan* variety grown at dryland due to seedlings had experienced early exposure of As phytoavailability in soil throughout the paddy life cycle. Accumulation of As in upper part of paddy plants cultivated at dryland were already detected at 2 months old (Figure 1). Paddy at early part of the life cycle are usually grown on wetter months and are harvested at drier months implicates the drier the soil environment, the more efficient As phytoavailability at sampling locations due to the physical and chemical properties of the soil.

Several paddy genotype have been identified as potential Cd hyperaccumulator (Duan *et al.*, 2017, Takahashi *et al.*, 2016). Nevertheless, Cd was not traced in soil and any parts of *Pandasan* paddy cultivated at flooded field during growing and seed production life cycle phase (Table 1). Although Cd was traced in soil and in *Pandasan* paddy cultivated at dry soil, translocation of Cd in the plant from root to grain is low as no trace of Cd was detected in the leaves and grain (Figure 1).

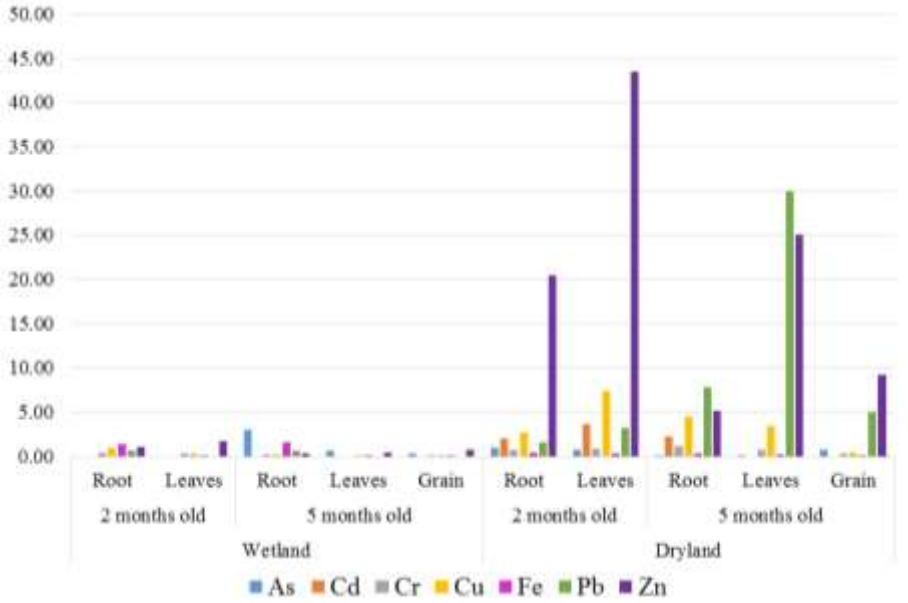

Figure 5 Enrichment factor of trace elements distribution in different parts of Pandasan paddy variety cultivated in wet and dry soil at two different sampling stages of the paddy life cycle.

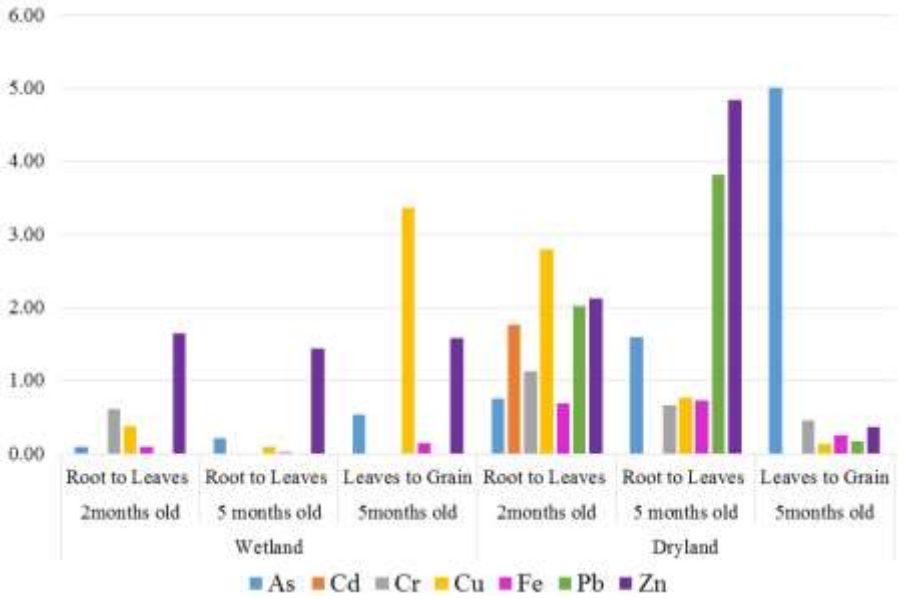

Figure 6 Translocation factor of heavy metal in Pandasan paddy variety from root to grain at two stages of life cycle phases: 2 months old (growing phase) and 5 months old (harvest phase) cultivated in different water availability environment conditions.

On well-drained agriculture areas, *Pandasan* paddy variety have the tendency to accumulate more trace elements of Pb, Zn and Cu in its biomass which is not used for consumption such as the stem and leaves (Figure 1). Thus, *Pandasan* can be a good candidate for phytoextraction of agricultural area in Crocker range which soil is not water-logged. However, pre-caution must be taken if *Pandasan* paddy is cultivated at dry area with high concentration of arsenic and lead as these two elements can translocate from root to grain in similar fashion like Zn which is highly mobile as well in this paddy genotype (Figure 2). Brown rice have higher content of As compared to polished rice (Meharg *et al.,* 2008). Concentration of As and Pb in rice grain cultivated at dry soil was above the permissible limit of Malaysia Food Regulation 1985 (Table 1). *Pandasan* paddy cultivated at flooded field was safer for consumption as none of the selected trace elements concentration exceeded the permissible limit of Malaysia Food Regulation 1985. For safer consumption, rice grain yielded from dry land could be prepared as polished rice to reduce heavy metal toxicity risk. On the other hand, rice grain yielded where heavy metal phytoavailability is low at water logged soil can be prepared as brown rice for more rich nutrient content.

Unchecked usage of pesticides or herbicides in the surrounding area can cause deterioration of productive land and long term health impact (Mohammed & Makame, 2015). Currently, slashing and burning is the common traditional method to clear up agricultural land (Hanafi *et al.,* 2009). Burning land with the aid of petrol can indirectly leave traces of Pb. A good method of disposing rice straws are required for successful reduction of heavy metal in agricultural land after *Pandasan* paddy had been cultivated considering rice straw utilization is low in Malaysia (Rosmiza *et al* 2014). Paddy waste also can be potentially utilized to clean up heavy metal by biosorption (Kumar et al 2017). Symptoms of Zn and Pb morphophytotoxicity showing on upper part of *Pandasan* paddy plant can be monitored as an alert of high bioaccumulation of these trace element on well-drained agricultural land as *Pandasan* paddy can be a candidate for lead and zinc bioindicator. Trace element uptake characteristics discoveries in *Pandasan* paddy plant can encourage multi-functions purposes not only as food resource but in soil remediation and biomonitoring activities.

CONCLUSIONS

Pandasan paddy plant's trace element uptake varies when cultivated on different soil environment at field sites. Trace element phytoavailability is more efficient in dry soil compared to wet soil of the studied field. Health risk of heavy metal toxicity can be reduced if *Pandasan* paddy is cultivated in water-logged soil compared to dry soil. Apart as a food resource for the farmers, *Pandasan* paddy can also contribute in heavy metal soil remediation for reducing Pb and Zn on well-drained agricultural land.

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ECOSYSTEM SERVICES WITHIN A KEY SUBTROPICAL REGION AFFECTED BY THE YACYRETA DAM IN PARAGUAY

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ABSTRACT

One of the most striking features of the ecosystem concept is that their components, through natural structures and processes, render ecological functions, which are valued by society. The objective of this research was to identify and describe ecosystem services provided by the Natural Reserve Yacyretá, in accordance with the goal of Paraguay's National Law Nº 3,001 of 2006 of conservation, protection, recovery and sustainable development of national natural resources and biological diversity through fair, timely and adequate valuation and payment for ecosystem services. The study was conducted during an environmental monitoring campaign in October 2016 in the reserve, using an ecosystem services checklist constructed based on the classification provided by *The Economics of Ecosystems and Biodiversity*. This reserve is part of the conservation, protection and compensation actions carried out by the Yacyretá Binational Entity in the area of influence of the dam constructed between Paraguay and Argentina for the Hydroelectric Power Station Yacyretá. Its purpose is the protection of ecosystems, communities of biological elements that, due to their rareness, fragility, importance or singularity deserve a special assessment. This reserve has a high representativeness of the resources of the Ñeembucú eco-region, and the presence of two ecosystems scarcely represented in Paraguay, such as forests of arary (*Callophyllum brasiliense*) and a small formation of vegetated dunes. The reserve provides all four categories of ecosystem services, directly and indirectly. These results will be practical for stablishing conservation strategies to update its management plan and assess access to the system of valuation and payment for ecosystem services.

Key words: *Conservation. Ecosystem services. Yacyretá dam.*

INTRODUCTION

An ecosystem is the basic unit of nature (Tansley, 1935) that includes both the biotic and abiotic components of a given area (Constanza et al, 1997) and their interrelationships (Beichler et al, 2017). One of the most striking features of the ecosystem concept is that their components, through natural structures and processes, render ecological functions. With an anthropocentric approach, they

produce benefits for people (de Groot et al, 2002; MEA, 2005). Therefore, ecosystem services, according to The Economics of Ecosystems and Biodiversity (2010), are referred to as the direct and indirect contributions of ecosystems to human well-being. The flow of ecosystem services to society is often studied in the environmental economics field, since it provides a quite comprehensive tool for decision-makers to value and even monetize ecosystem services. For example, since 2006, Paraguay has a valuation and payment system for ecosystem services established by National Law Nº 3,001. Its goal is to promote conservation, protection, recovery and sustainable development of national natural resources and biological diversity through fair, timely and adequate valuation and payment for ecosystem services. This law considers ecosystem services all human activities of management, conservation and recovery of ecosystem functions that benefit people directly and indirectly.

The Yacyretá Dam of Hydroelectric Power Plant Yacyretá, is one of the three projects that provide electricity to Paraguay. It is a binational project with Argentina, located on the Paraná river, between the cities of Ayolas (Paraguay) and Ituzaingó (Argentina), 300 Km Southwest of Asunción and 1,000 Km North of Buenos Aires. Because of its several environmental impacts and as compensation for the loss of natural environments caused by the flooding for the filling of the reservoir, the Yacyretá Binational Entity (EBY, for its initials in Spanish) has executed a series of actions tending to the protection of different representative habitats and species in their area of influence. According to what is stated by National Law Nº 3,001, these biodiversity reserves could access the system of valuation and payment for ecosystem services. The purpose of these reserves is the protection of ecosystems, communities or biological elements that, because of their rareness, fragility, importance or singularity deserve a special assessment, according to Paraguay's National System of Protected Wild Areas (SINASIP, for its initials in Spanish) and 1994 National Law Nº 352 of Protected Wild Areas. Particularly, EBY has settled in Paraguay a total of 19,256 hectares in biodiversity reserves,of which 6,300 correspond to the Natural Reserve Yacyretá. This reserve has a high representativeness of the resources of the Ñeembucú eco-region, and there can be found forests in flooded soil with dominance of arary (*Callophyllum* brasiliense and small formations of vegetated dunes. These have a special relevance because of their rareness since these ecosystems appear scarcely represented in Paraguay, respectively only found in the Yacyretá Island and in a very limited area in the Boquerón department, on the North border with Bolivia. Despite all conservation and management efforts, there are several critical points, especially of anthropic pressure caused by poaching, arson, transit of vehicles and people, nearby human settlements and solid waste. It should be noted that the nearby area is subject of study for rice production through the exploitation of the Aguapey stream basin, where the reserve is located. The management plan of the Natural Reserve Yacyretá (2005), has objectives related to conservation of biological diversity, environmental education and research, among others, but none directly related to the identification of ecosystem services. Therefore, this paper

intended to identify and describe ecosystem services provided by this reserve within this key subtropical region affected by the Yacyretá dam, in accordance with National Law Nº 3,001 of 2006.

MATERIALS AND METHODS

This research had a descriptive approach, according to Hurtado de Barrera (2000), as it intends to identify and describe ecosystem services provided by the Natural Reserve Yacyretá, in accordance with the Paraguay National Law Nº 3,001 of 2006. There are no previous similar studies in the reserve that consider ecosystem services, even if they are necessary for a better understanding and management of these natural resources and for considering access to the valuation and payment system. Paraguay's system includes forest protection and management, reforestation and other activities that mitigate greenhouse gases, protection services for water resources, springs, wetlands and watersheds, protection services of species and ecosystems, care of natural landscapes, and soil protection and recovery services, to name a few. Even though it provides a general concept and description of ecosystem services, it lacks the structure that the de Groot et al (2002), MEA (2005) or TEEB (2010) have. For this reason, this study was conducted considering their classification, as can be seen on the next table.

Table 1. Categories of ecosystem services

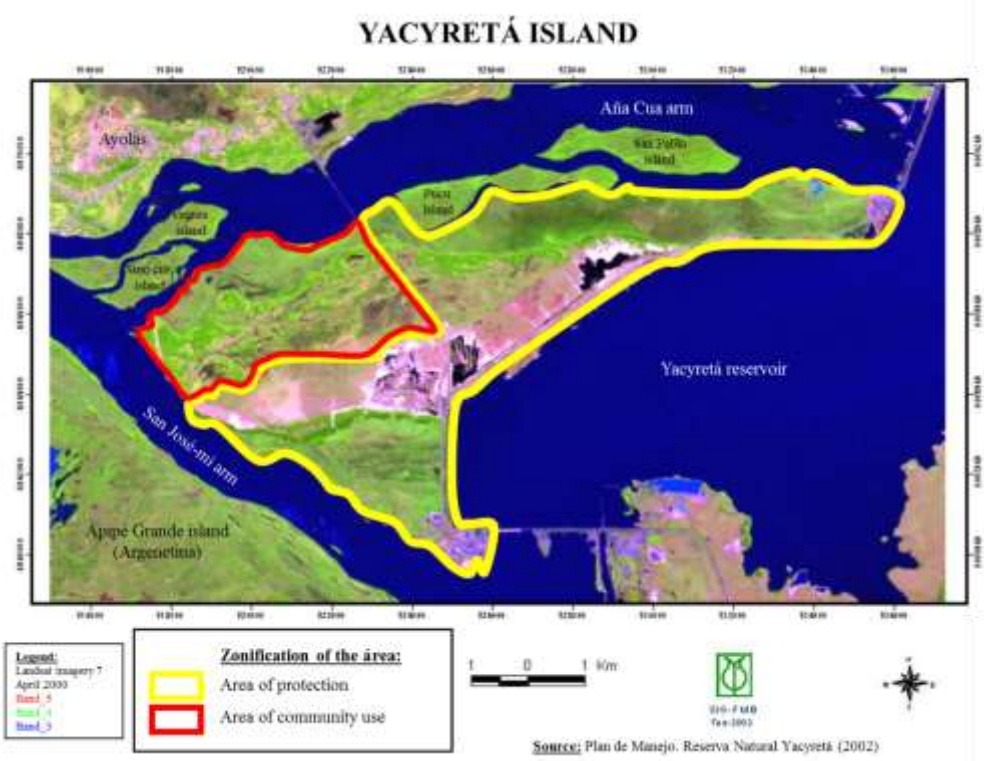
Provisioning services	**Regulating services**	**Cultural services**	**Habitat services**
They describe the material outputs from ecosystem.	Capacity to regulate essential ecological processes and life support systems through biospheric processes.	Include the non-material benefits people obtain from contact with ecosystems	Ecosystems provide living spaces for plants and animals. They also maintain a diversity of different breeds of plants and animals

Adapted from de Groot (2002), MEA (2005) and TEEB (2010)

Provisioning services include food, raw materials, fresh water and medicinal resources. Regulating services incorporate local climate and air quality regulation, carbon sequestration and storage, moderation of extreme events, wastewater treatment, erosion prevention and maintenance of soil fertility, pollination, and biological control. Cultural services involve spiritual enrichment, cognitive development, reflection, recreation, and aesthetic experience. Supporting of habitat services cover soil formation, photosynthesis, primary production, nutrient cycling, and water cycling. All four categories were considered for the arrangement of a checklist of ecosystem services, which was applied in a monitoring campaign in October 2016. The observations were later organized, analysed, integrated and consolidated in a report of results, which are presented in the next section of this paper.

The study was conducted in the Natural Reserve Yacyretá, created in 2009 by EBY Resolution Nº 11,716, located in the Yacyretá island, in the Ayolas district

(Misiones department), West of the trace of the Yacyretá dam. It is delimited North by the Aña Cuá arm and South by the San José-mi arm. These details can be found in the next map:



Map 1: Location and distribution of the Yacyretá Island – Translated from EBY (2002)

RESULTS AND DISCUSSION

Provisioning ecosystem services

Even though hunting and fishing are prohibited in the reserve, the ecosystem presents a high production of edible plants and animals such as wild mammals, fishes, birds, fruits, and exotic items like bird's nests. According to the Management Plan (2005), there were registered specimens of kui`î or porcupine (*Sphiggurus spinosus)*, lobope (*Lontra longicaudis*), apere´a (*Cavia aperea*), tatu poju (*Euphractus sexcinctus*), tatu hû (*Dasypus novemcinctus*), akuti (*Dasyprocta azarae*), teju guasu (*Tupinambis rufescens*); birds like the white heron (*Ardea alba*), mytû (*Penélope obscura*) and *taguato´i* (*Buteo magnirostris*). Different species of primates can also be found in this ecosystem, standing out the karaja (*Alouatta caraya*) and the ka'i paraguái, also known as Capuchine monkey (*Cebus apella*), aside from several species of reptiles and amphibians. In addition to the mentioned species, several forests and shrub species can be used as raw

materials for food, firewood and charcoal, pasture and forage, nectar and honey, medicines, crafts, industrial and ornamental uses, among others.

Table 2: Species of flora of ecological and economic importance in the Yacyreta Natural Reserve

Scientific name	Common name	Scientific name	Common name
Acrocomia aculeata	Coco	*Myrciaria baporeti*	Yvaporoity
Calophyllum brasiliense	Arary	*Pterogyne nitens*	Yvyra ro
Rheedia brasiliensis	Pakuri	*Helietta apiculata*	Yvyra ovi
Xylopia brasiliensis	Yvyra katu	*Enterolobium contortisiliquum*	Timbó
Tabebuia pulcherrima	Tajy sa‟y ju	*Gleditsia amorphoides*	Yvope
Tabebuia impetiginosa	Tajy	*Jacaratia spinosa*	Jacaratia
Tabebuia heptaphylla	Tajy	*Bumelia obtusifolia*	Yvyra hu
Butia jatai	Jata‟i	*Chrysophyllum gonocarpum*	Aguai
Syagrus romanzoffiana	Pindó	*Guadua angustifolia*	Takuarusu
Bahuinia forficata	Pata de buey	*Ocotea spp*	Laurel
Brachiaria sp.	Pasto	*Nectandra spp*	Laurel
Sebastiania brasiliensis	Yvyra kamby	*Inga uruguensis*	Inga guasu
Senecio brasiliensis	Agosto poty	*Holocalyx balansae*	Yvyra pepe
Trichilia catigua	Katigua pyta	*Elionurus muticus*	Espartillo
Trichilia clausenii	Guatambi mi	*Pseudananas ananoides*	Ananá de monte
Trichilia pallens	Katigua moroti	*Bromelia balansae*	Karaguatá
Trichilia pallida	Cedrillo	*Tillandsia sp*	Clavel del aire
Sapindus saponaria	Palo jabón o casita	*Rhipsalis sp*	Cactus
Sorocea bonplandii	Ñandypa mi	*Oncidium sp*	Orquidea
Maclura tinctoria	Tatajyva	*Myrciaria baporeti*	Yvaporoity
Eugenia uniflora	Ñangapiry	*Pterogyne nitens*	Yvyra ro
Hexachalamys edulis	Yva hai	*Helietta apiculata*	Yvyra ovi

Source: EBY (2005)

The extraction of these floral species is also prohibited in the reserve. Amid these floral species, there are several medicinal plants, such as tajy (*Tabebuia heptaphylla*), pata de buey (*Bahuinia forficata*), palo jabón or casita (*Sapindus saponaria*), ñangapiry (*Eugenia uniflora*), ybaporoity (*Myrciaria baporeti*) and aguai *(Chrysophyllum gonocarpum*). About freshwater production services, several artificial lagoons can be found in the reserve stocking rainfall water. These lagoons formed as result of sand exploitation for the construction of the dam.

Habitat or supporting services

Alongside the Yacyreta reservoir, there are large extensions of land with conserved areas for protection of different species. Natural ecosystems have an essential role in regulating and maintaining ecological processes as well as maintenance of genetic resources, so areas like the vegetated dunes provide habitats for plants and animals. While the collection of species for commercial use is prohibited, during this study, wild species like lobo pe (*Londra longicaudis*) and kui'i or porcupine (*Sphiggurus spinosus*) were observed. This species can be used for meat and several other flora species have uses in medicine and industry, like it was mentioned before.

Regulating ecosystem services

Although the reserve represents a 4.5 % of forest cover of the Ayolas district, according to the National Forestal Institute (2016), this could be considered relatively low. Despite this, the reserve sequestrates and stores carbon from the atmosphere, which can provide regulation of local climate and air quality. The atmosphere of the vegetated dunes ecosystem appears to have no signs of pollution in its natural state, since there are no industries or densely populated urban areas in the proximities. Regarding UV radiation, it was observed an increase due to the reflection of the artificial lagoons.

In respect to moderation of extreme events, the presence of forests in around 50 % of the total area of the reserve helps to absorb rainfall, flooding, and balances local climate because of the carbon sequestration and storage. Around the reserve, there are wetlands of approximately 1 Km wide and 15 Km long that work as firewalls against natural fires and arson.

About pollination service, butterflies, bees and hummingbirds were observed acting as pollinators. This is an essential service for the ecological balance of the reserve. It ensures the life cycle of plants and genetic variability needed to adapt to changes in the environment.

Natural ecosystems control a high percentage of all potential crop pests and disease vectors. These can be easily controlled in a pristine environment, but the Yacyreta Natural Reserve has a continuous flow of visitors that can cause risks for the ecosystem, due to the hauling of pests and diseases, plant manipulation, and constant trampling.

The reserve's soil is of basaltic origin, with very little organic material, and degradation of rocks due to human intervention. Since the area is covered with native and exotic shrub and herbaceous vegetation, the soil is protected from the erosive action of wind and water. Grass species, through the action of their roots, act as structures for soil particles and help avoid direct impact from raindrops.

Vegetation and biota have a role in removing and decomposing nutrients and foreign components. The vegetated dunes are subject to constant visits from tourists and students, visits that transport foreign components such as dust, yerba mate, hot and cold water, and buses can bring seeds, dirt and mud from other places. Despite these characteristics, there could not be found visible solid waste like plastic bottles and paper cups, or even smaller particles like yerba mate.

Cultural ecosystem services

The vegetated dunes offer opportunities for different forms of nature tourism, for instance, trekking, controlled or sectored eco-tourism, and sightseeing that can also provide environmental education to visitors. The presence of many important species of birds offer opportunities for bird watching. Other species of mammals, like capybaras and karajas, can be exploited for guided visits and photographic safaris. The reserve can also be visited for educational and scientific observation purposes.

It should be noted that the vegetated dunes in Yacyretá Natural Reserve represent a unique ecosystem in Paraguay. Pieces of driftwood can be found and extracted

from the river and artificial lagoons, which then can be used by artisans to create wooden sculptures. Other ornamental resources like seeds, feathers, claws, teeth could be exploited. Other plants and animal products could be sold as souvenir or as collection items, such as orchids and butterflies, which could attract tourists and collectors. The reserve's objectives are clear about conservation, though. Despite this, its uniqueness could inspire books, documentaries, paintings or even advertising, activities that don't have extractive purposes.

According to the reserve's guides, the vegetated dunes do not receive visits for spiritual, religious or historic experiences, only for recreation and tourism. However, this ecosystem provides a proper environment to value natural and even religious heritage. Regarding the latter, the reserve's natural characteristics and distance from populated areas can make it an ideal place for meditation.

CONCLUSIONS

The environmental monitoring campaign identified potential uses of available resources and helped comprehend ecosystem's intrinsic value as generator of ecosystem services. These aspects could be included or considered to update the current management plan, especially bearing in mind the conservation objectives of the reserve. The vegetated dunes in the Yacyretá Natural Reserve provide regulating, provisioning, cultural, and support ecosystem services. All four categories could be observed, some directly, like food and raw materials, and others indirectly, such as medicinal resources. This shows that this ecosystem needs more research in this field, maybe considering each ecosystem service separately. A carrying capacity study is urgent since this ecosystem is part of an eco-tourism circuit in the Yacyreta Natural Reserve. If these visits surpass its carrying capacity, the provisioning of ecosystem services could be endangered.

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PHENOLIC COMPOSITION AND ANTIOXIDANT ACTIVITY OF GREEN-SOLVENTS-BASED EXTRACTS OF RED ONION WASTES

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ABSTRACT

Onion represents one of the most important crop, based on its production, consumption and high pharmacological potential supported by its chemical composition. Phytochemicals of phenolic structure, in particular anthocyanins, are among the strongest antioxidant compounds with large biological and industrial applications. Because their conventional extraction involves the use of high amounts of polar organic solvents, there is a strong requirement for development of new strategies based on greener solvents. The aim of the present paper was to extract valuable compounds of polyphenolic structure from red onion wastes using deep eutectic solvents, as green strategy for their isolation. In addition, the total antioxidant activity as measured by ferric reducing antioxidant capacity assay was investigated. Extraction performed at 40^{o}C in the selected solvent system was optimized for two parameters, solvent/sample ratio and extraction time. The obtained results showed the highest content of anthocyanins and antioxidant activity at a solvent/sample ratio of 30/1 and the extraction time of 90 min, at 40^{o}C. Regarding the total phenolics and flavonoids, the optimal extraction parameters were 20/1 solvent/sample ratio and 90 min, at 40^{o}C. Compared to conventional extraction in organic solvent, the values of the antioxidant activity were similar, while those of the content of bioactive compounds were higher in extracts obtained in ethanol solution. By further optimization of the extraction process, the obtained products may find useful application in obtaining natural ingredients intended for different purposes.

Keywords: *Red onion wastes, deep eutectic solvents, phenolics, anthocyanins, antioxidant activity.*

INTRODUCTION

Onion is an important crop, proved by its significant production, consumption and, not at least, potential valorization through extraction of biologically active compounds, such as fructo-oligosaccharides, minerals, fiber, and flavonoids. Apart the edible parts of onions, a lot of wastes, *e.g.* skins, outer fleshy scales and roots,

are produced mainly during industrial processing. As considerable non-edible parts of onions are generated, research has been focused on the potential valorization of its components for the development of bioactive ingredients. Phytochemicals of different chemical structures may provide human health benefits. The extraction of such molecules from various plant matrices is the first and most significant step for obtaining effective and safe final products. The most efficient extractive technology is that one which generates high amounts of targeted compounds, preserves as much as possible their biological activity and provides no negative environmental impact. Phytochemicals of phenolic structure, in particular anthocyanins, are among the strongest antioxidant compounds with great technological applicative potential (Kowalczyk *et al.*, 2003). Traditionally, they are extracted by using polar organic solvents or acidified solvent solutions which favor the stabilization of the red flavylium cation (Giusti and Wrolstad 2001; Revilla *et al.*, 1998). Several extraction parameters, such as solvent, solvent/sample ratio, time, temperature, and pH have been optimized such as to recover high amounts of anthocyanins, but also to avoid their degradation (Castañeda-Ovando *et al.*, 2009). The use of large amounts of organic solvents for the extraction of natural products may generate a negative environmental impact and may lead to small impurities in the final extract. Modern extraction techniques have been investigated and reported, such as ultrasound-assisted extraction (UAE) (Vinatoru, 2001**),** pressurized liquid extraction (PLE) (Ju and Howard, 2003; Feuereisen *et al.*, 2017) and supercritical fluid extraction (SPE) (Bleve *et al.*, 2005; Maran *et al.*, 2014). The current requirement for decreasing the impact of solvents moved the scientific research and the industry strategies toward new greener solvents, with low cost, reduced energy consumption, high solute solubility, selectivity and environmental compatibility. Deep eutectic solvents which are mixtures of compounds with lower melting points have been proposed as alternative solvents for green extraction being also biodegradable and having pharmaceutical accepted toxicity (Abbott *et al.*, 2007). Particular compositions of such solvents provide not only acceptable extractability but also the ability to stabilize some compounds with limited stability under various conditions, such as anthocyanins (in particular cyanidin) (Dai, 2013). The aim of the present paper was to get optimal extraction of phenolic compounds (anthocyanins, phenolics, flavonoids) under different parameters (solvent/solid ratio, extraction time) by using deep eutectic solvents. Solid red onion wastes (dry skins) were selected as potential chip source of valuable compounds, in particular red pigments (anthocyanins). In addition, the total antioxidant activity as measured by ferric reducing antioxidant capacity FRAP was determined.

MATERIALS AND METHODS

Plant material and chemical reagents: Commercially red onions (*Allium cepa* L.) were purchased from Romanian local market. Only dry skins were collected and grounded into powder before extraction processes (Grindomix GM 200, Retsch, Germany). The moisture content was determined at 105^{o}C using the moisture

analyzer (MAC 210/NP Radwag, Poland). Chemical reagents of analytical grade were used.

Extraction procedure: Deep eutectic solvents, 1, 2-propanediol, choline chloride and water were prepared at a molar ratio of 1/1/1 as described by Dai (Dai *et al.*, 2013). The following extraction parameters were applied: solvent/sample ratio (30/1 and 20/1) and three predetermined extraction times (30, 60 and 90 min) at 40^{o}C. Similar experiments were run with 70% (V/V) ethanol solution, for comparison. Further, samples were centrifuged at 4000 rpm for 10 min, filtered on 0.45 μm cellulose acetate membrane filters and diluted with water. The prepared crude extract was used for the determination of phenolics, anthocyanins, flavonoids, and total antioxidant activity.

Total phenolics: The content of total phenolics was determined spectrophotometrically according to the Folin-Ciocalteu method (Singleton and Rossi, 1965). The Specord 200Plus UV–Vis spectrophotometer (Analytik Jena, Germany) was used. The results were expressed in milligram of gallic acid equivalents per 100 g dry mass (mg GAE 100g^{-1} DM).

Total anthocyanins: The content of total anthocyanins was determined spectrophotometrically by the pH differential method (Giusti and Wrolstad, 2001). The content was expressed as milligram cyanidin-3-O-glucoside (Cyn-3-O-G) equivalents per 100 g dry mass (mg 100g^{-1} DM).

Total flavonoids: The content of total flavonoids was determined using the aluminum chloride colorimetric method (Kumar *et al.*, 2008). The content was expressed as milligram quercetin equivalents per 100 g dry mass (mg 100g^{-1} DM).

Antioxidant assay using Ferric Reducing Antioxidant Power (FRAP): The total antioxidant activity of crude extracts was determined by the ferric reducing ability assay described by Benzie (Benzie and Strain, 1996). The results were expressed as milligram ascorbic acid per 100 g dry mass (mg 100g^{-1} DM).

Statistical analysis: Data presented are the average of two replicates, expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

The choline chloride-based eutectic solvent (choline chloride/1,2-propanediol/ water) was tested for extraction of the main antioxidant compounds of polyphenolic structure from red onion wastes. 1,2-propanediol is widely used as solvent in pharmaceutical formulations, cosmetics and food products. In the solvent system of organic salt, 1,2-propanediol acts as hydrogen donor while water is added for the decrease of viscosity. The mixture has a melting point lower than the individual components. The presence of many –OH groups, polarity and viscosity of deep eutectic solvents favors the extraction of bioactive compounds of phenolic structure due to the formation of H-bonds (Dai *et al.*, 2013). In order to further decrease the viscosity, extraction was conducted at 40^{o}C. In our study, water was added to choline chloride and 1,2-propanediol (9% V/V) as it increases the extraction efficiency; it has been shown that higher water content (>50%) drastically decreased the yield of extraction probably due to the rupture/weakening

of the interactions between deep eutectic solvents and bioactive compounds (Bi *et al.*, 2013). The contents of the main antioxidant compounds extracted from red onion skins using choline chloride/1,2-propanediol/water are presented in Figures 1-3. As noticed, extractability of the investigated compounds increased with time of extraction, from 30 to 90 min., at 40°C. No significant difference was found between solvent/solid ratio regarding the content of total anthocyanins. However, mean values showed that 30/1 might be more efficient. Phenolic compounds were extracted with highest yield using a solvent/sample ratio of 20/1 and extraction time of 90 min. Similar results were obtained for flavonoids.

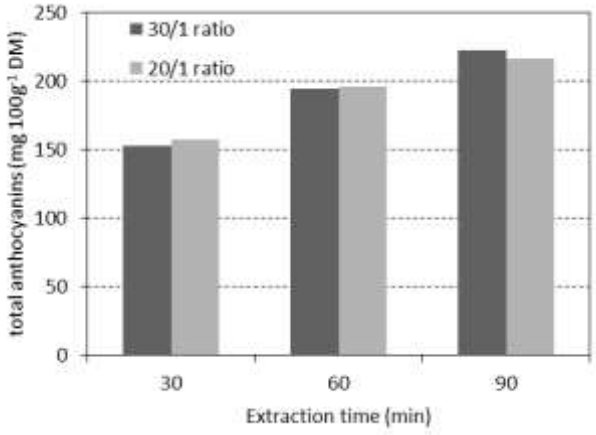

Figure 1. Total anthocyanins content of the crude extract of red onion skins in deep eutectic solvent according to different parameters.

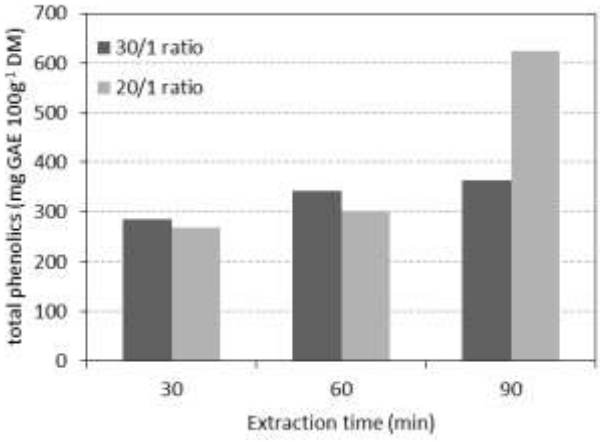

Figure 2. Total phenolics content of the crude extract of red onion skins in deep eutectic solvent according to different parameters.

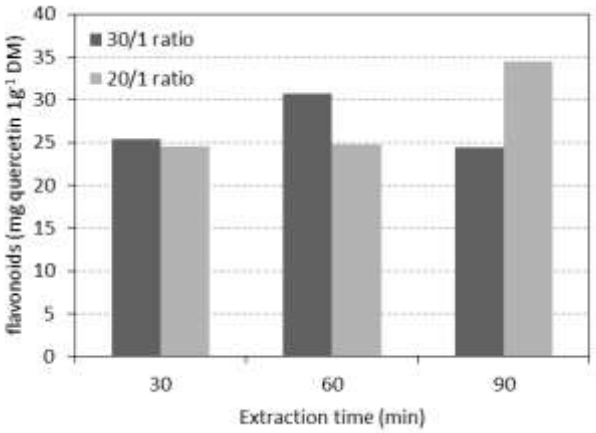

Figure 3. Total flavonoids content of the crude extract of red onion skins in deep eutectic solvent according to different parameters.

The contents of total anthocyanins and flavonoids were four time higher in extracts prepared under similar conditions but with conventional solvent (70% ethanol solution), while significant differences were found between total phenolics in extracts with deep eutectic solvents and ethanol solution. This might be due to the lower polarity of polyalcohol in the prepared deep eutectic solvent compared to other hydrogen donors, such as organic acids which may also improve extraction of some compounds (anthocyanins) due to the low pH.

To our knowledge there is no report on extraction of anthocyanins from red onion skins with the aid of deep eutectic solvents and on the evaluation of their antioxidant activity. However, literature reports the use of deep eutectic solvents based on other hydrogen donors (sugars, organic acids) which were tested for anthocyanins extraction from wine lees (Bosiljkov *et al.*, 2017). These authors found a promising combination of using natural deep eutectic solvents with ultrasonication for an efficient extraction of anthocyanins. Regarding phenolic compounds, the study of Fernández *et al*. showed that individual phenolics (caffeic acid, rutin, quercetin, tyrosol) were extracted from dry scapes and umbels from onion seed production using lactic acid/glucose/water assisted by ultrasonication (Fernández *et al*., 2017).

The results regarding the total antioxidant activity of the crude extracts as measured by FRAP assay are presented in Figure 4.

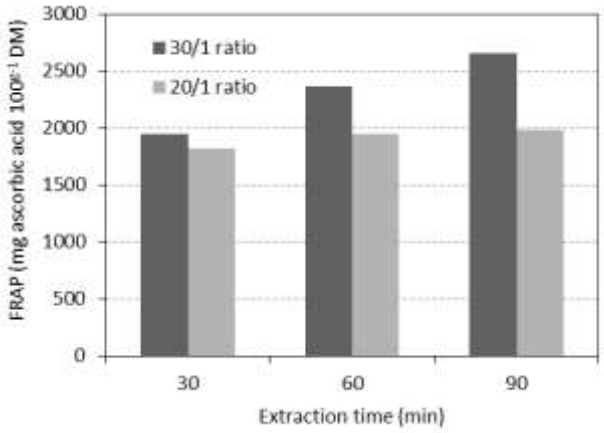

Figure 4. Ferric reducing antioxidant power (FRAP) of the crude extract of red onion skins according to different parameters.

The mean value of FRAP activity obtained using deep eutectic solvents (2120.09 mg ascorbic acid $100g^{-1}$ DM) was similar to that of extracts under conventional extraction with 70% ethanol solution (2781.13 mg ascorbic acid $100g^{-1}$ DM) highlighting the great potential of these solvents for eco-friendly extraction of plant antioxidants compounds. However, special attention has to be paid to practical aspects of deep eutectic solvents extraction and there is still room for further research in this area.

CONCLUSIONS

Horticultural and industrial wastes generated from red onion processing contain valuable compounds which may be exploited as ingredients for various purposes. Such approach of managing wastes was hereby combined with the testing of a green extraction technology of polyphenolic-based compounds.

The results of the study of the influence of extraction parameters showed that increased time (90 min) gave efficient extraction yield of bioactive compounds at 40^{o}C. Promising results were obtained regarding the total antioxidant activity of the crude extracts, which were similar to those obtained under conventional extraction with ethanol solution (30/1 ratio, 90 min, 40^{o}C).

Deep eutectic solvents emerged as new green solvents with promising results on recovery of important compounds from plant materials. Nevertheless, future research is required to evaluate different compositions of deep eutectic solvents such as to increase the extractability of anthocyanins, phenolics or flavonoids from red onion wastes compared to conventional organic solvents.

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COMPARATIVE BIODIVERSITY BETWEEN NO-TILL AND CONVENTIONAL TILL ON A CROP ROTATION

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ABSTRACT

The technological development that agriculture has suffered in recent decades has affected biological diversity in agricultural fields. In particular, the life on the soil surface, that is mainly constituted by invertebrates. This loss of biodiversity entails the disappearance of natural processes that this organisms support. For this reason, it is necessary to implement agronomic management that reduces impacts on agricultural soils. One of these alternatives is no-tillage system, which is characterized by the absence of soil ploughing and the maintenance of crop residues on soil surface. In this sense, the present work has compared the existing biodiversity in a crop rotation (sunflower-wheat-legume) between plots under no-tillage and plots under conventional tillage systems. With this objective, insects, arachnids, crustaceans and myriapods have been captured through pitfall traps. Four plots have been sampled, 2 under no-tillage and 2 under conventional tillage. In each plot have been placed 4 sampling areas, consisting of 5 pitfall traps each. The catches made have shown higher biodiversity values in no-tillage compared to conventional tillage. These differences have been significantly higher in terms of number of species captured and with respect to the biodiversity indices of Margalef, Simpson and Shannon. However, the increases in number of individuals captured has not been significant as well as the uniformity indices of Pielou and Simpson.

Keywords: *Biodiversity, Arthropods, no tillage, biodiversity indices, pitfall traps.*

INTRODUCTION

Traditionally, the agricultural activity has led to the realization of a series of tasks on the ground. The technological development that agriculture has suffered in recent decades, has strengthened these tasks, decisively affecting the biodiversity that inhabits them. This biodiversity located in the soil is not reduced to the edaphic profile, there is a part of it that lives on its surface, called epigeous fauna, which includes mostly invertebrates. The taxonomic composition within the

invertebrates has in the arthropods (insects and arachnids, although also some crustaceans and myriapods) the majority group, both in abundance and in diversity.
The loss of the biodiversity of the faunal community that lives on the ground entails the disappearance of the processes that it sustains. In general, a rich and diverse epigeous fauna provides a greater number of benefits on the soil than the damages it can generate. In fact, high values of biodiversity in the surface of the soil benefit agricultural production, as shown by the works with arthropods on cereal crops by Edwards and Lofty (1978). Among the benefits, it stands out in the first place the maintenance of a complex trophic chain, which sustains the stability of the ecosystem and prevents the proliferation of pests through predatory organisms. And secondly, the decomposition and availability of nutrients carried out by the action of saprophagous fauna.
In view of the need to avoid or reduce the pernicious effects caused by conventional agriculture on biodiversity, the implementation of sustainable agronomic measures, such as those derived from the application of Conservation Agriculture (CA), is necessary. The reduction of the tillage of the ground and the implantation of vegetal covers, allowing to increase the general biodiversity that occurs in them (Cantero, 2005). These measures have repercussions from the general scope of the group of macroarthropods to more specific taxonomic groups, such as the coleoptera (beetles). In the work of House and Parmelee (1985), the biodiversity observed under direct sowing conditions is compared with that existing in conventional crops, detecting higher values of the same in the first case.
The main component among the macroarthropods of the soil surface is that of insects, where the most diverse life forms and life models are found. Among the different groups of insects on the surface of the soil, it has been shown that the application of CA positively affects the populations of coleoptera, and more specifically, staphillid and carabid beetles. While the diversity and density of carabids decreases in those areas where agricultural practices are most intense (Holland and Luff, 2000), species richness and diversity of staphylinids are increased with reduced tillage and fewer applications (Krooss and Schaefer, 1998). Shearin et al. (2007) in herbaceous crops, calculated a 50% reduction in the activity of coleoptera under tillage conditions over non-tillage, while Fereres (1997), Andersen (1999) and Marasas et al. (2001) also demonstrated the population benefit of carabids and staphylinids in soils without tillage. As for the ants, it seems that the implementation of CA measures does not have such a clear impact. Occasionally, a population increase in tillage crops has been detected with respect to non-tillage crops (Campos et al., 2002). This circumstance may be due to the greater effort that ants must make in the search for food under tillage conditions due to an environment with less availability of resources, which causes a greater frequency in the capture of individuals in the samplings.
Arachnids are the organisms on the surface of the soil that have the greatest benefits in the application of CA measures, since farming negatively affects their presence (Castro et al., 1996). Similarly, it has been proven that soil plowing also negatively affects their populations (Ekschmitt et al., 1997).

In the case of the myriapods (millipedes and centipedes), studies have also been carried out (Wolters and Ekschmitt, 1997) that testify to their sensitivity to the alteration imposed by soil tillage management.

Other arthropods that are also present on the soil surface of cultivated land are crustaceans. Among them, highlights the cochineal moisture, whose abundance in crops under CA, can become pests, as reflected Alfaress (2002) in bean crops under no-tillage conditions of some regions of North America.

With the objective of verifying at a practical level the influence on the biodiversity of the implementation of CA measures in a farm, the existing differences in macroarthropod biodiversity (between plots in conventional tillage (CT) and plots with direct sowing have been studied (NT). In this way it is intended to corroborate the benefits for biodiversity of the application of NT, as well as to verify that the proposed methodology can be applied to evaluate, in an easy and fast way, the evolution of the biodiversity of a certain crop when the tillage is reduced.

MATERIAL AND METHODS

The study of the macroarthropods biodiversity (arthropods with more than 2 mm thickness) has been carried out in a farm of the Rabanales University Campus, located in the vicinity of the city of Córdoba. In this farm, treatments with NT and conventional tillage CT have been carried out during 4 agricultural campaigns. Specifically, the farm has been divided into 4 rectangular plots, 2 for each treatment (Fig. 1).

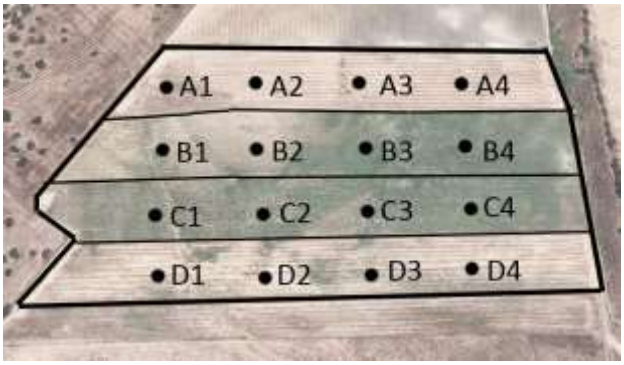

Fig. 1. Parcels of study in the Rabanales farm and sampling points.

There has been a rotation of sunflower-wheat-legume during the four seasons. In the campaign in which the data collection was carried out, plots A and D are planted with wheat, while plots B and C are planted with sunflower. In terms of management, plots A and B have been cultivated using CT, while C and D have been cultivated with NT. In order to have representative blocks for the statistical analysis in each plot, four sampling points have been established, distributed equally along each of them (Fig. 1). Therefore, for each of the treatments (CT and

NT) a total of 8 sampling points have been established, 4 in a plot planted with wheat and another four in a plot planted with sunflower (Table 1).

Table 1. Characteristics of each sampling point.

Sampling	Management	Crop in the last campaign	Bloq
A1	CT	wheat	1
A2	CT	wheat	2
A3	CT	wheat	3
A4	CT	wheat	4
B1	CT	sunflower	1
B2	CT	sunflower	2
B3	CT	sunflower	3
B4	CT	sunflower	4
C1	NT	sunflower	1
C2	NT	sunflower	2
C3	NT	sunflower	3
C4	NT	sunflower	4
D1	NT	wheat	1
D2	NT	wheat	2
D3	NT	wheat	3
D4	NT	wheat	4

Each sampling point is composed, in turn, of five drop traps (plastic cups placed at ground level with preservative liquid) arranged in a straight line and separated by 1 meter of distance (Fig 2.), in a similar direction to the larger side of the plot. As a preservative liquid in each of the fall traps, 40 ml of a 10% dilution of ethylene glycol was poured.

The traps were kept for 4 days. In the collection of the samples, all the individuals corresponding to a sampling point were united in a same bottle, for later analysis in the laboratory.

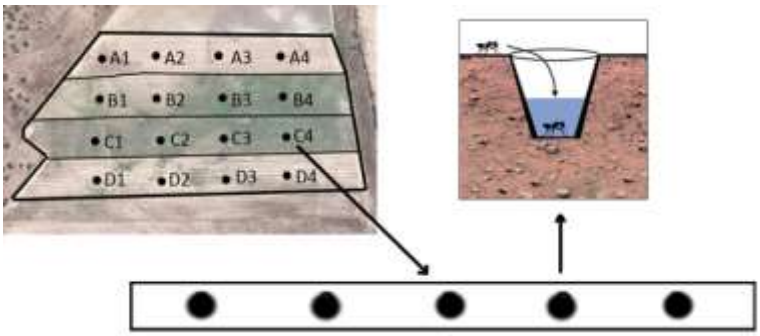

Fig. 2 Schematic of the placement of the fall traps.

Once the samples had been taken to the laboratory, the contents of each vial were filtered through a 2 mm light sieve. The arthropods retained in the sieve have been visually checked and separated by pseudospecies. That is, those individuals with a

similar appearance have been cataloged within the same pseudospecies. This methodology generates a margin of error, being able to consider within the same pseudospecies individuals of different species with very similar appearance. Or, catalog in pseudospecies different individuals that, being of the same species, have a different aspect due to sex or stage of development. But it is a good approximation for a comparative study of biodiversity like the one contemplated here, avoiding a great work of taxonomic determination in the laboratory. Above all, considering the high number of individuals captured (1730).

Once both the number of pseudospecies and the number of individuals for each pseudospecies in each sampling point have been quantified, a biodiversity calculation has been made in each one of them through several indexes of biodiversity and equitability (Table 2).

Table 2. Indices studied

Index	Simbols	Fórmule
Margalef´s Biodiversity Index	I	$I = (S - 1) / Ln N$
Simpson´s Biodiversity Index	D	$D = 1 / (Pi)^2$
Shannon´s Biodiversity Index	H	$H = - (Pi * Ln Pi)$
Pielou's Uniformity index	J	$J = H / ln (S)$
Simpson´s Uniformity index	E	$E = D / S$

S is the number of species. Pi the proportion of individuals of species i with respect to the total of individuals N. That is, the relative abundance of species i: ni / N. nor is the number of individuals of species i. N is the number of all individuals of all species.

With the aim of observing the statistical significance of the results, an analysis of variance was performed for each of the indices studied, through the Statistic 9 software. Specifically, by means of the variance analysis option from a design in blocks. Subsequently, a Tukey HSD Test was performed at $p \leq 0.05$, to verify the existence of significant differences between the results of biodiversity in plowing and direct sowing.

RESULTS AND DISCUSSION

Table 3 shows the data of the number of pseudospecies and individuals in each sampling point. In addition, the results of the calculation of the different indexes of biodiversity and equitability that have been studied are included.

Table 3. Results obtained in each sampling.

Muestreo	Pseudospecies	Individuals	Margalef	Simpson	Shannon	U. Pielou	U. Simpson
A1	24	246	4.1777683	3.4482051	1.6701279	0.525519	0.1436752
A2	20	263	3.4098124	2.175331	1.2718646	0.4245588	0.1087665
A3	24	202	4.3328636	4.0344077	1.9013572	0.5982772	0.1681003
A4	20	113	4.0191329	3.8894303	1.8659154	0.6228578	0.1944715
B1	14	42	3.478103	7.8053097	2.3089872	0.8749287	0.5575221
B2	23	56	5.4653609	10.594594	2.7068928	0.8633065	0.4606345
B3	15	74	3.2527389	7.7344632	2.2695377	0.8380707	0.5156308
B4	17	91	3.5469958	3.251276	1.8034993	0.6365561	0.1912515
C1	18	41	4.5778026	7.5381165	2.4457261	0.8461631	0.4187842
C2	17	33	4.5759946	10.572815	2.5908075	0.9144413	0.6219303
C3	25	53	6.0448955	12.160173	2.8349691	0.8807326	0.4864069
C4	25	65	5.7493467	8.6048879	2.6434541	0.8212352	0.3441955
D1	26	101	5.4169766	5.8458452	2.4179419	0.7421333	0.2248402
D2	37	111	7.6440745	5.9781659	2.6481964	0.7333859	0.161572
D3	32	137	6.3008374	10.799194	1.9321299	0.5574948	0.3374748
D4	30	102	6.2703071	5.5281615	2.2469939	0.6606479	0.184272

The analysis of variance was made to the data in Table 3, which allowed us to study the existence of differences between the different managements, with the results obtained being those shown in Fig. 3.

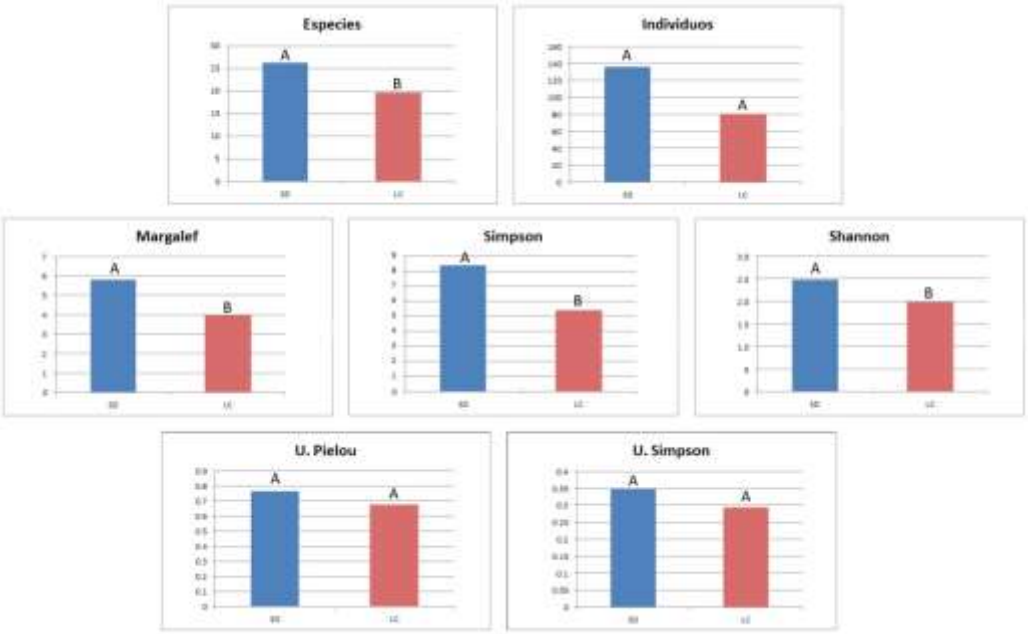

Fig. 3. Statistical significance of the results. The height of each column shows the average of the data obtained for each treatment. The different letters indicate significant differences compared to the Tukey test at $p \leq 0.05$.

As can be seen in Fig. 3, there are significant differences in NT with respect to CT in the data referring to species and in the biodiversity indices. On the other hand, as regards individuals and uniformity indices, there is no such significance. In any case, in all the graphs, higher values are observed in NT than in CT. According to what is stated in Martella et al., 2012, where it is indicated that the values for the Shannon index are between 1.5 and 3.5 normally, the macroartropod biodiversity in the NT plots, with an average close to 2 , 5, can be considered intermediate, while for CT, with a value lower than 2, it can be considered as low. The results obtained in the uniformity indices indicate that the distribution of the individuals among the different species is similar in NT and CT. These indices show if the individuals are equitably distributed among the species or there are some much more dominants. For example, the Pielou´s Uniformity index of (according to Martella et al., 2012) adopts values between 0 and 1. Number 1 indicates that all species are equally abundant and 0 indicates the absence of uniformity. Therefore, an average value close to 0.7 for NT and CT, indicates that there is a high level of equality in the distribution of individuals between species for both managements. The results show that the application of Direct Seeding measures in a rotation of arable crops has a positive effect on macroarthropod biodiversity. In fact, the data are significantly superior to the conventional tillage in the three indices studied (Margalef, Simpson and Shannon), as well as in the number of species found, there being a correspondence with what was presented in Cantero (2005) and House and Parmelee (1985).

CONCLUSIONS

It is corroborated that the methodology followed seems to be propitious to evaluate, easily and quickly, the effect on the macroarthropod biodiversity in a crop when applying NT, being able to be used to indicate a greater environmental sustainability of the same with respect to another in CT.

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IDENTIFICATION OF IN SILICO MIRNAS IN FOUR PLANT SPECIES FROM FABACEAE FAMILY

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ABSTRACT

Plant microRNAs (miRNAs) are small non-coding RNAs, about 21-24 nucleotides, which have critical regulatory roles on growth, development, metabolic and defense processes. Their identification, together with their targets, have gained importance in exploring their parts on functional context, providing a better understanding of their regulatory roles in critical biological processes. With the advent of next-generation sequencing technologies and newly developed bioinformatics tools, the identification of microRNA studies by computational methods has been increasing. In the presented study, we identified some putative miRNAs for *Cicer arietinum*, *Glycine max*, *Medicago truncatula* and *Phaseolus vulgaris* genomes. We also provided the similarity between those organisms regarding common/different miRNAs availability throughout their genomes. According to the data, the highest similarity was found between *Glycine max* and *Phaseolus vulgaris*. We also investigated the potential targets of putatively identified miRNAs for each organism. We analyzed which miRNA families were expressed *in silico*. We also showed the representation (copy number of genes) profile of predicted putative miRNAs for each organism. Since most of the food products and animal feeds consist of Fabaceae family members as it is mentioned above, these findings might help to elucidate their metabolic and regulatory pathways to use them efficiently in biotechnological applications and breeding programs.

Keywords: microRNA, *Cicer arietinum*, *Medicago truncatula*, *Glycine max*, *Phaseolus vulgaris*.

INTRODUCTION

Recently, the sufficiency of food demands becomes a critical issue since the increasing world population, drastic changes in climate and the a/biotic stress factors has threated the sustainability of agricultural production. Therefore, there is an immediate need to develop new farming technologies and biotechnological applications (Akpinar et al., 2012).

As one of the most critical and useful development, next-generation technologies help us to unravel the complex genomes of organisms in addition to having a significant impact on reducing the cost, time and required effort compare to the previous methods such as Sanger sequencing. Based on different sequencing technologies, various computational tools and analysis methods were developed. Computational microRNA identification studies on plant genomes have been increased and contributed to the recent literature efficiently. MicroRNAs (miRNAs) are small, about 21-24 nucleotides, endogenous non-coding RNAs that play various roles in plants. They are derived from the stem-loop structure, and some specific enzymes modify them. Plant microRNAs control the expression of genes encoding multiple transcription factors, stress-responsive elements, and the other proteins have roles in growth, development and physiological properties (Rogers and Chen, 2013). Computationally identified miRNAs has reached to the successful means, and some new miRNAs were identified experimental methods. These experimentally identified miRNAs had roles on abiotic stresses due to drought, salinity, heat, cold or phosphorous deficiency or biotic stresses. Currently, computational miRNA prediction is based on two approaches: 1.) Homology-based for conserved miRNA identification 2.) Some other algorithms which use support vector machine by setting some characteristics for pre-miRNA structure (Zhang et al., 2006).In our study, we used the 'homology-conserved' method to predict some putative miRNAs via using in-house Perl scripts (Avsar and Aliabadi, 2017a; Avsar and Aliabadi 2018). Legumes belong to the Fabaceae family are essential nutritional sources for foodstuffs and animal feeds. Their rich protein, starch content, oil, fiber content and the high efficiency of nitrogen fixation properties make Legumes highly valuable in the cropping cycle, and therefore they account for one-third of global primary crop production (Mantri et al., 2013). In this study, four different legume genomes were studied due to their economic importance and/or their suitable model features: *Cicer arietinum* (chickpea), *Glycine max* (soybean), *Medicago truncatula* and *Phaseolus vulgaris* (common bean). The genomes of these species have been completely sequenced, and they are available in NCBI. We putatively identified miRNAs for each species, and we compared their microRNA atlas to each other as well as the model organism "*Medicago truncatula*." These findings may help us to have a better understanding of the roles of miRNAs in abiotic stress, the miRNAs involved in symbiosis and nutrition homeostasis.

MATERIAL AND METHODS

Reference miRNAs and Datasets: Currently available mature miRNA sequences belong to Viridiplantae (8,496 sequences and 73 plant species) were downloaded from miRBase release 21 (Kozomara and Griffiths-Jones, 2013). miRBase corresponds to 4,802 unique mature miRNA sequences, and these mature miRNAs were used as a query in homology-based *in silico* miRNA identification. Legumes genomes were retrieved from NCBI. All plant assemblies were downloaded from

NCBI (GenBank accessions: GCA_000004515.3, GCA_000499845.1, GCA_000331145.1, GCA_000219495.2*)*.

Homology conservation approach for miRNA identification: The prediction was employed using two previously developed, in-house Perl scripts: SUmirFind and SUmirFold[1]. In the first step of homology-based miRNA prediction, BLAST+ stand-alone toolkit, version 2.2.25 (Camacho, 2009) was used for detection of database sequences with homology (mismatch cutoff parameter set to <=3) to previously known plant mature miRNAs (Avsar and Aliabadi, 2015). In the second step, UNAFold version 3.8 was used with parameters optimized to include all possible stem-loops generated for each miRNA query to obtain secondary structures of predicted miRNAs. Perl scripts eliminated hairpins with multi-branched loops, with inappropriate DICER cut sites at the ends of the miRNA-miRNA* duplex, or with mature miRNA sequence portions at the head of the pre-miRNA stem-loop.

Representative miRNAs (gene copy number) on target genomes: The miRNA gene copy numbers were identified based on the output data from SUmirFold process mentioned in section Homology conservation approach for miRNA identification. Identical miRNA families that were resulted from the similar miRNA stem-loop sequences were eliminated to avoid over-representation.

Expressed Sequence Tag (EST) analysis, miRNA targets and target annotations of predicted genomic miRNAs: For EST analysis, the pre-miRNA sequences were retrieved, and the duplicate sequences were removed to prevent over-representation. By using the BLAST+ stand-alone toolkit, version 2.2.25, pre-miRNA sequences were blasted to EST sequences specific to each organism obtained from NCBI (Avsar and Aliabadi 2017b). The strict criteria (above the threshold as 98% identity and 99% query coverage) were used for the identification of expressed miRNA families. Mature sequences were identified, and duplicates were removed. By using online web tool, psRNA, the mature query sequences were blasted against to EST sequences. The resulting file was used for gene ontology analysis by using Blast2Go software (Conesa and Götz, 2008). The predicted mature miRNA sequences were also searched in miRBase database website to confirm their experimentally validated targets.

RESULTS AND DISCUSSIONS

Putative miRNAs in Fabacea family members: We predicted as a total of 198 putative miRNA families. Out of 198 putative miRNA families 42, 150, 44, 41 putative miRNA families in *Cicer arietinum*, *Glycine max*, *Medicago truncatula* and *Phaseolus vulgaris* genomes, respectively and 42 common miRNAs were found between all organisms (Table 1).

[1]http://journals.plos.org/plosone/article/file?type=supplementary&id=info:doi/10.1371/journal.pone.0040859.s003

Table 1. Putative miRNA families identified for each organism. Ca: *Cicer arietinum*, Gm: *Glycine max*, Mt: *Medicago truncatula*, Pv: *Phaseolus vulgaris*

Ca	Gm				Mt	Pv	Common
miR1130	miR160	miR2606	miR4406	miR9765	miR172	miR160	miR160
miR1511	miR1507	miR403	miR4410	miR1526	miR1030	miR1510	miR1510
miR1514	miR1508	miR4340	miR482	miR2089	miR1120	miR1512	miR1512
miR156	miR1509	miR4342	miR4996	miR2218	miR1128	miR1514	miR1514
miR157	miR1510	miR4343	miR5030	miR3522	miR1439	miR1515	miR1527
miR159	miR1512	miR4344	miR5034	miR4355	miR1525	miR1527	miR156
miR160	miR1513	miR4345	miR5035	miR4394	miR159	miR156	miR157
miR162	miR1514	miR4346	miR5037	miR4413	miR2118	miR159	miR159
miR164	miR1516	miR4347	miR5038	miR477	miR2218	miR162	miR162
miR165	miR1517	miR4348	miR5041	miR5205	miR2592	miR164	miR164
miR166	miR1520	miR4349	miR5042	miR5370	miR2593	miR165	miR165
miR167	miR1521	miR4350	miR5043	miR5763	miR2599	miR166	miR166
miR168	miR1527	miR4352	miR530	miR5773	miR2600	miR167	miR167
miR169	miR1531	miR4356	miR5372	miR5774	miR2601	miR168	miR168
miR170	miR1535	miR4359	miR5376	miR9742	miR2602	miR169	miR169
miR171	miR156	miR4360	miR5377	miR9743	miR2603	miR170	miR170
miR172	miR157	miR4361	miR5378	miR9766	miR2605	miR171	miR171
miR2099	miR159	miR4363	miR5380	miR9767	miR2606	miR172	miR172
miR2111	miR162	miR4364	miR5667		miR2607	miR2111	miR2111
miR2118	miR164	miR4365	miR5670		miR2608	miR2118	miR2118
miR2218	miR166	miR4366	miR5775		miR2619	miR2119	miR2119
miR2618	miR167	miR4367	miR5780		miR2627	miR2218	miR2218
miR2630	miR168	miR4368	miR5784		miR2629	miR319	miR2606
miR319	miR169	miR4369	miR862		miR2630	miR390	miR2630
miR390	miR171	miR4371	miR9723		miR2636	miR391	miR319
miR393	miR172	miR4372	miR9730		miR2652	miR393	miR390
miR394	miR1863	miR4373	miR9732		miR2655	miR394	miR393
miR395	miR2107	miR4374	miR9734		miR2670	miR395	miR394
miR396	miR2109	miR4376	miR9735		miR2671	miR396	miR395
miR397	miR2111	miR4380	miR9736		miR319	miR397	miR396
miR398	miR2118	miR4382	miR9739		miR399	miR398	miR397
miR399	miR2119	miR4384	miR9745		miR482	miR399	miR398
miR5037	miR319	miR4387	miR9746		miR5161	miR403	miR399
miR5205	miR390	miR4388	miR9749		miR5205	miR4376	miR403
miR5213	miR393	miR4390	miR9752		miR5249	miR4407	miR4376

miR5281	miR394	miR4391	miR9753	miR5281	miR4416	miR482
miR5287	miR395	miR4392	miR9754	miR5282	miR482	miR5037
miR529	miR396	miR4393	miR9755	miR5287	miR5037	miR5205
miR530	miR397	miR4395	miR9756	miR530	miR529	miR5281
miR5741	miR398	miR4399	miR9757	miR5554	miR530	miR5287
miR6275	miR399	miR4401	miR9761	miR5561	miR829	miR529
miR6440	miR408	miR4402	miR9762	miR5745		miR530
	miR5281	miR4404	miR9763	miR7696		
	miR529	miR4405	miR9764	miR7701		

According to the results, *G.max-P.vulgaris* had more common miRNAs (34) whereas *M.truncatula-P.vulgaris* (8) shared the least amount of common miRNA families. The miRNA repertoire depends on genome size so *G.max* (about 980 MB) may have more miRNA families on its genome than the other organisms: *P.vulgaris* (about 521 MB), *C.arietinum* (about 530 MB), *M.truncatula* (about 412 MB). For each organism, putative miRNA families gave detailed information including conserved miRNA ID, miRNA* sequence, pre-miRNA stem sequences, calculations related to MFE, MFEI and GC%. Lower MFE values show the high stability of predicted miRNAs. Minimal folding free-energy index (MFEI) values which were calculated using MFE and GC% values differentiate miRNAs with typically higher MFEIs (>0.67) from other types of cellular ssRNAs for which MFEIs were previously characterized; transfer RNAs (0.64), ribosomal RNAs (0.59), and mRNAs (0.62–0.66) (Schwab et al., 2005).

Representation of putative miRNAs on genomes: In here, we used unmasked data to find representatives of miRNA families on genomes. According to this analysis, for *P.vulgaris* and *C. arietinum*, highly representative miRNA families, miR171, was similar. However, for *G.max* and *M.truncatula*, miR1520 and miR5281 families were profoundly found, respectively (Figure 1). Low representations of miRNA families (less than ten copy number) were calculated, but they are not included in the graphs since they might be contamination or 'young-miRNAs.' On the other hand, the highest number of hits might be caused by repetitive elements because most of the transposable elements were domesticated into microRNA genes (Li et al., 2011).

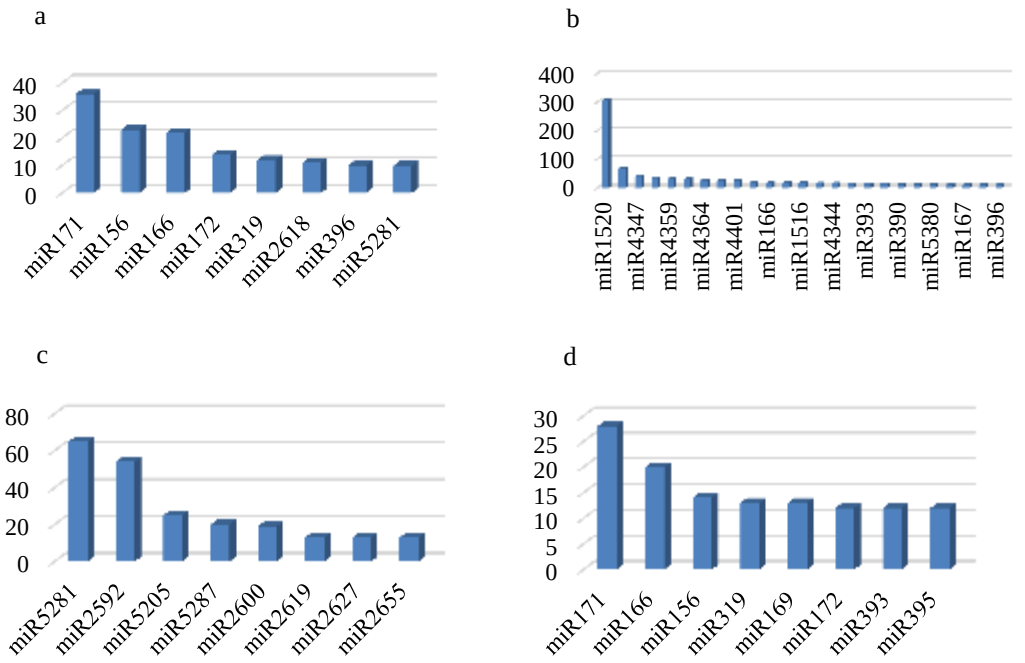

Figure 1. Representative miRNA families on genomes. a: *C. arietinum*, b: *G.max*, c: *M.truncatula*, d: *P.vulgaris*

Target prediction, gene ontology and expression analysis of identified miRNAs: We identified targets of putative miRNAs and their possible functions in the cell. As biological processes mechanisms, putative miRNA targets were mostly found in metabolic and cellular processes. Only *G.max* putative miRNAs targeted the genes found in the cellular component organization or biogenesis processes (Figure 2a). Putative miRNA targets were identified in almost all cellular components, however, for the macromolecular complex part, only *C.arietinum* and *M.truncatula* had low percent of target sequences (Figure 2b). Molecular functions of putative miRNA targets were also detected for all organisms. Catalytic activity and binding functions had the highest percentage whereas structural molecule activities of targets were only identified for *C.arietinum* putative miRNAs (Figure 2c).

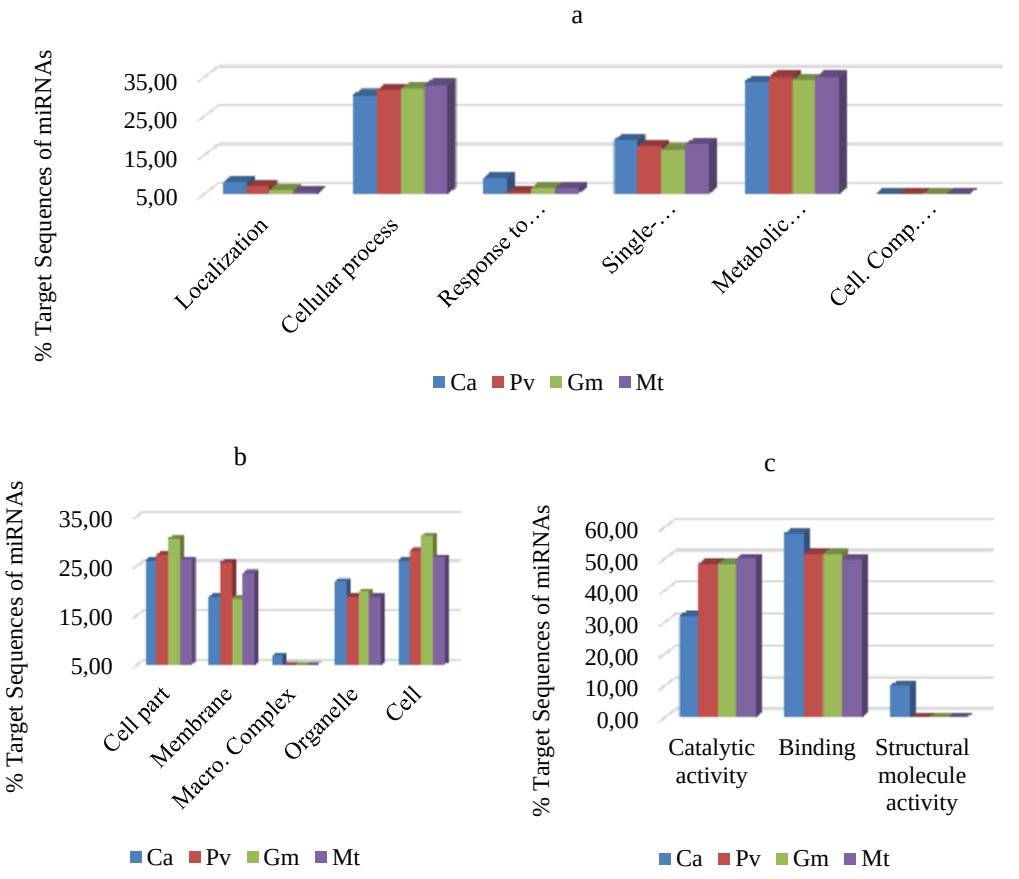

Figure 2. a: Biological processes of miRNA targets, b: Cellular component of miRNA targets, c: Molecular functions of miRNA targets. Ca: *Cicer arietinum*, Gm: *Glycine max*, Mt: *Medicago truncatula*, Pv: *Phaseolus vulgaris*

We also analyzed the expression of the predicted miRNAs *in silico*. For this purpose, the pre-miRNA sequences from each miRNA families were selected and blasted against to EST databases of each organism. In *C.arietinum*, only miR156 families had high homology to different EST sequences in GenBank. In *G.max*, we found 34 different miRNA families (miR1507, miR1508, miR1509, miR1510, miR1514, miR1520, miR156, miR160, miR162, miR166, miR167, miR168, miR171, miR172, miR2089, miR210, miR2109, miR211, miR2218, miR319, miR3522, miR394, miR395, miR396, miR398,miR399, miR403, miR408, miR482, miR4996, miR5038, miR529, miR5372, miR5667) showed a high homology to EST sequences. In *M.truncatula*, eight putative miRNAs were identified as miR159, miR2118, miR2218, miR319, miR399, miR482, miR5281, miR7696. For *P.vulgaris*, miR151, miR167, miR168, miR171, miR211, miR2118, miR221 and

miR399 families were given positive results according to the threshold mentioned in Materials and Methods section. For EST databases retrieved from NCBI, *C.arietinum* had the least amount of EST sequences whereas *G.max* had the most amount of EST sequences. Therefore, this may affect the identified *in silico* expressed miRNA families that show variation between the organisms.

CONCLUSIONS

MicroRNA discoveries provide us an opportunity to understand better complex regulatory systems in plants and in particular those involved in a/biotic stress conditions. This study helps research community to develop stress-tolerant crops by breeding programs. Additionally, unraveling the roles of miRNAs in the symbiotic relationships of legumes in overcoming several important agriculturally limiting environmental stresses is of high priority. Our findings may also help researchers to understand the regulatory roles of putative miRNAs in Fabaceae species which show genetic diversities and those which was analyzed by some molecular markers (Avsar, 2011). For the future studies, widely distributed and highly conserved miRNA families should be experimentally validated. These miRNAs are known as essential elements in different mechanisms ranging from abiotic stress tolerance to seed development. Furthermore, performing evolutionary studies for close relatives to understand their similarities/differences based on the miRNA repertoires and the functions of these putative miRNAs inside the organisms are valuable.

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A STUDY ON PLANT HEIGHT CONTROL OF *IRIS* FLOWERS

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ABSTRACT

We investigated the effect of paclobutrazol as preplant bulb soaks on plant height of *Iris x hollandica* cv. 'Frans Hals' and 'Blue Magic'cultivars which were grown in pots. Bulbs of iris were soaked into gibberellin inhibitor paclobutrazol solution at 0, 15, 30 ppm before planting. Effect of paclobutrazol on the flowering time, flower diameter and length, leaf length, plant height, flower life, and chlorophyll content of leaves were determined. The shortest plant height was obtained from the 'Blue Magic' cultivar treated with 30 ppm paclobutrazol which gave plants with 11.3 cm, 68% shorter than untreated control. 'Frans Hals' cultivar treated with 30 ppm paclobutrazol was 20.9 cm and 50% shorter than control. In 'Blue Magic' and 'Frans Hals'cultivars the lower dose of 15 ppm paclobutrazol were also effective on height control with 11.8 and 21.5 cm plant height, respectively. This gibberellin inhibitor also shortened the leaf length of iris cultivars. Paclobutrazol treatments resulted in higher chlorophyll content per unit area in the leaves. The highest chlorophyll content (57.00 CCI) was obtained from the 'Blue Magic' iris treated with 30 ppm paclobutrazol, while the control plants had 32.70 CCI chlorophyll in their leaves. Chlorophyll content of 'Frans Hals' treated with 30 ppm paclobutrazol were 52.87 CCI, while control plants of this cultivar were 28.80 CCI. Plants applied with paclobutrazol resulted with smaller flower diameter compared to the control plants of both cultivars. The smallest flower diameter was obtained from 30 ppm paclobutrazol treatment with 38.83 mm in 'Blue Magic' iris while the control of this cultivar was 99.63 mm. The flower diameter of 'Frans Hals' cultivar treated with 30 ppm paclobutrazol was 109.1 mm, while the control one was 112 mm.

Keywords: *Paclobutrazol, bulb soak, Iris x hollandica, plant height.*

INTRODUCTION

Iris x hollandica from the Iridaceae family is perennial plant and known as 'Dutch Iris. *Iris x hollandica* crossed two varieties of *Iris xiphium* (var. *praecox* from Spain and var. *lusitanica* from France) with *Iris tingitana* from North Africa (Hekstra and Boertjes 1968; Okubo and Sochacki; 2012). The majority of the Irises are the rhizomatous species. However the bulbous species are the most important commercially. Most of the bulbous cultivars are Dutch irises and they are bulbous

irises (Okubo and Sochacki, 2012). Irises are horticulturally important plants and have been used for centuries both as ornamental plants and a source of making perfumes (Köhlein, 1987; Fancescangeli, 2009). Bulbous iris species are produced commercially in greenhouses as cut flowers and potted plants (Le Nard, 1983; Fancescangeli, 2009). When cut flower species are produced in pots a problem of these cultivars of great commercial interest is that the stalk is longer. It is important that maintain a harmonious relationship with the container and plant growth (Krug, 2004; Fancescangeli, 2009). In addition elongation can continue after production at consumer conditions with low light. These reasons make it difficult to use as indoor plants (Çelikel et al., 2016). Therefore, plant height control is important for maintaining compactness and aesthetically pleasing appearance, as well as preventing damage during transportation and marketing due to stem elongation (Çelikel et al., 2016). We may control plant height either by physical methods with environmental factors (light, temperature, etc.), or by chemical methods with growth regulators mostly gibberellin inhibitors (Demir and Çelikel, 2013; Çelikel et al., 2016). These inhibitors are paclobutrazol, flurprimidol, ancymidol, uniconazole, chlormequat chloride and daminozide (Currey and Lopez, 2017).

A concentration of 20 mg L^{-1} paclobutrazol was found sufficient to achieve harmonious plants with the containers in 'Casablanca' and 'Professor Blaauw' *Iris x hollandica* cultivars and these plants were shorter 41 to 44% compared to the control plants (Fancescangeli, 2009). It was reported that growth regulators such as ancymidol, paclobutrazol, flurprimidol act to reduce gibberellin levels in the plant thereby causing shorter plants (Miller, 2010a). Paclobutrazol or uniconazole pre-plant dips were effective in controlling height of 'Anna Marie' hyacinths (Miller, 2002). Plant height of 'Carlton' narcissus cultivar was controlled by flurprimidol and paclobutrazol (Miller, 2010b). Paclobutrazol preplant bulb soaks significantly controlled 'Tete a Tete' narcissus plant height during greenhouse forcing at concentration ≥ 150 ppm. Flurprimidol as bulb soaks at 20 mg L^{-1} controlled the plant height of 'Pink Pearl' hyacinth cultivar cultivars (Krug et al., 2006a).

Effects of paclobutrazol were not investigated on plant height of *Iris x hollandica* cv. 'Frans Hals' and *Iris x hollandica* cv. 'Blue Magic' cultivars before. Therefore, we investigated the effects of paclobutrazol as preplant bulb soaks on plant height and other properties of *Iris x hollandica* cv. 'Frans Hals' and *Iris x hollandica* cv. 'Blue Magic' grown in pots.

MATERIAL AND METHOD

Iris x hollandica cv. 'Frans Hals' and *Iris x hollandica* cv. 'Blue Magic' bulbs with circumference of 8-10 cm from Asya Lale (Konya, Turkey) were used in this study. Bulbs were soaked into paclobutrazol (PBZ, 25% Cultar; Syngenta) of 0, 15, 30 ppm for 30 min before planting. Bulbs were allowed to air dry and were planted into a 15 cm diameter plastic pots (1.6 volume) containing soil, peat and perlite (1:1:1) as one bulb per pot on the day of treatment (3 November 2016). Plants grown in a polyethylene covered greenhouse were irrigated as hydroponic drip irrigation system.

Flowering time and flower life: Flowering time was determined as number of days from planting time to opening of the flower (when petal color is visible). Flower life was calculated as the number of days from the opening of the flower to the wilting of the flower.

Plant height and leaf length: The plant height (from the pot rim to the uppermost of the inflorescence) and leaf length (the longest leaf) were started to measure respectively 172 days (24 April) and 37 days (20 December) after planting, when they were started to emerge. Measurements were made weekly.

Chlorophyll content: Chlorophyll content of leaves was measure by chlorophyll meter (Apogee) at anthesis time. It was determined as Chlorophyll content index (CCI).

Flower diameter: The flower diameter was measured by a caliper at anthesis time.

Data Analysis: Data were tested by two way analysis of variance (ANOVA). The study was conducted with 10 replications. The obtained data were analyzed statistically by using the SPSS package program. The mean and standard error ($\overline{X} \pm S\overline{x}$) values were determined. Differences between means were separated by Duncan's multiple range test ($P \leq 0.01$).

RESULTS AND DISCUSSION

Flowering time and flower life: PBZ delayed the flowering time of irises. There was significant ($P \leq 0.01$) difference among the application. The latest flowering was obtained from 15 and 30 ppm PBZ with 184 days in 'Frans Hals' cultivar, while control 182 days. PBZ also delayed flowering in 'Blue Magic' cultivar. Flowering was accrued 179 and 180 days after planting in 'Blue Magic' iris treated with 15 and 30 ppm PBZ, while control was 176 days. A delay was observed in some *Iris* cultivars in the visible appearance buds in plants treated with PBZ (Francescangeli, 2009). The application of PBZ delayed the appearance of the flower color in *Petunia* (Francescangeli and Zagabria, 2009). Flurprimidol application caused flowering delay of *Ornithogalum saundersiae* (Salachana and Zawadzińska, 2013). Blázquez et al., (1998) reported that the gibberellin class of plant hormones has been implicated in the control of flowering in several species. It was reported that exogenous GA_2 promote the switch from vegetative growth to flowering in a variety of plants by Wilson et al., 1992. Therefore the gibberellin inhibitors of paclobutrazol used in this study, effected flowering. Treatments affected the duration of the cycle. Flowering time was not affected by lower rates of flurprimidol, but it was slightly delayed when flurprimidol was applied at higher doses in 'Mona Lisa' lily cultivar (Pobudkiewicz and Treder, 2006). In our study gibberellin inhibitors of paclobutrazol didn't affect the flower life of iris cultivars. There was no difference among the applications in flower life of irises. However, there was difference ($P \leq 0.01$) in flower life between cultivars (Table 1).

Table 1. The effects of paclobutrazol (PBZ) on flowering time and flower life of *Iris x hollandica* cv. 'Frans Hals' and *Iris x hollandica* cv. 'Blue Magic'

Mean ± Standard Error ($\overline{X} \pm S\overline{x}$)

Cultivar	Treatments	Flowering time (days)	Flower life (days)
Iris x hollandica cv. 'Frans Hals'	Control	182.4 ± 0.33 b	10.1 ± 0.46 a
	15 ppm PBZ	184.1 ± 0.46 a	10.1 ± 0.60 a
	30 ppm PBZ	184.3 ± 0.58 a	10.0 ± 0.30 a
Iris x hollandica cv. 'Blue Magic'	Control	176.4 ± 0.48 d	7.9 ± 0.58 b
	15 ppm PBZ	179.3 ± 0.75 c	7.5 ± 0.29 b
	30 ppm PBZ	179.5 ± 0.22 c	7.3 ± 0.21 b
Significance		0.000	0.000
Means of cultivars	'Frans Hals'	183.6 ± 0.71	10.07 ± 0.89
	'Blue Magic'	178.4 ± 0.47	7.57 ± 0.43
Significance		0.782	0.000

* Different letters in the same columns indicate differences among treatments according to Duncan multiple range test (1%).

In our study the PBZ applications decreased the plant height and leaf length (Table 2). There was significant ($P \leq 0.01$) difference among treatments for plant height (Table 2). The shortest plant height (11.3 and 11.8 cm) was obtained from 15 and 30 ppm paclobutrazol treatments, respectively, in 'Blue Magic' cultivar whereas the control plants were 35.6 cm (Table 2). 'Frans Hals' treated with 15 and 30 ppm paclobutrazol were 20.9 and 21.5 cm, while the control 41.5 cm, the longest one. 'Blue Magic' irises applied with gibberellin inhibitors of paclobutrazol were 68% (30 ppm PBZ) and 67% (15 ppm PBZ), 'Frans Hals' irises applied with gibberellin inhibitors of paclobutrazol were 50% (30 ppm PBZ), 48% shorter than control. Paclobutrazol substrate drenches control plant height of 'Tete a Tete' narcissus cultivar during greenhouse forcing (Krug et al., 2006b). Soil drenches of uniconazole retarded shoot and petiole elongation of *Brassaia actinophylla* (Wang et al., 1990). In another study flurprimidol, paclobutrazol and uniconazole suppressed height of 'Divine Cherry Red', 'Divine Scarlet Bronze Leaf' and 'Divine White Blush' *Impatiens hawker* cultivars (Currey et al., 2016). It was reported that paclobutrazol and uniconazole application reduced plant size and stem length of Kalanchoe 'Rako' (Lee et al., 2003). Uniconazole and flurprimidol are gibberellin inhibitors like paclobutrazol. Similarly in our study gibberellin inhibitors of paclobutrazol decreased the plant height of iris cultivars (Table 2).

The shortest leaf length (26.1 and 28.2 cm) was obtained from Blue Magic irises treated with 30 and 15 ppm PBZ while the control plants of 'Blue Magic' cultivar were 38.1 cm. The longest leaf length is 42.1 cm in untreated control of 'Frans Hals' cultivar. The leaf length of 'Frans Hals' treated with 15 and 30 ppm PBZ were 34.3 and 33.9 cm, respectively. There was significant difference among the application for leaf length (Table 2). It was reported that Topflor (flurprimidol) treatment shortened leaf length of hyacints cultivars (Miller 2010a). Uniconazole

foliar spray caused to reduction in leaf length and width of *Fuchsia x hybrida* (Kim, 1995). The leaf size of plants applied flurprimidol was smaller than the control plants in 'Mona Lisa' lily cultivar (Pobudkiewicz and Treder, 2006). The use of flurprimidol resulted with the shorter leaves in *Ornithogalum saundersiae* (Salachana and Zawadzińska, 2013) and *Zantedeschia aethiopica* (Gonzalez et al., 1999). Similarly in our study paclobutrazol were effective to shorten leaf length of irises during greenhouse production period.

Table 2. The effects of paclobutrazol (PBZ) on plant height and leaf length of *Iris x hollandica* cv. 'Frans Hals' and *Iris x hollandica* cv. 'Blue Magic'

Mean ± Standard Error ($\overline{X} \pm S\overline{x}$)

Cultivar	Treatments	Plant height (cm)	Leaf length (cm)
Iris x hollandica cv. 'Frans Hals'	Control	41.5 ± 1.07 a	42.1 ± 0.89 a
	15 ppm PBZ	21.5 ± 1.19 c	34.3 ± 0.90 c
	30 ppm PBZ	20.9 ± 0.82 c	33.9 ± 1.00 c
Iris x hollandica cv. 'Blue Magic'	Control	35.6 ± 0.43 b	38.1 ± 0.59 b
	15 ppm PBZ	11.8 ± 1.65 d	28.2 ± 0.74 d
	30 ppm PBZ	11.3 ± 0.68 d	26.1 ± 1.15 d
Significance		0.000	0.000
Means of cultivars	'Frans Hals'	27.97 ± 1.23	36.77 ± 0.58
	'Blue Magic'	19.57 ± 1.18	30.80 ± 0.89
Significance		0.003	0.009

* Different letters in the same columns indicate differences among treatments according to Duncan multiple range test (1%).

There was significant difference ($P \leq 0.01$) in chlorophyll content of leaves and flower diameter of 'Frans Hals' and Blue Magic' iris cultivars (Table 3). Paclobutrazol treatments caused an increase in chlorophyll content of leaves (Table 3). The highest chlorophyll content was obtained from 30 ppm paclobutrazol treatments both 'Blue Magic' (57 CCI) and 'Frans Hals' (52.9) cultivars while control plants was 32.7 CCI ('Blue Magic') and 28.8 CCI ('Frans Hals') (Table 3). In addition the lower doses (15 ppm) of paclobutrazol also increased the chlorophyll content of irises. Chlorophyll content of irises treated with 15 ppm paclobutrazol was 51.3 CCI ('Blue Magic') and 46.2 CCI ('Frans Hals'). The use of flurprimidol resulted plants with an increased relative chlorophyll content of *Ornithogalum saundersiae* (Salachna and Zawadzińska, 2013). Paclobutrazol application for cuphea and uniconazole application for petunia produced compact and high quality plants (Ahmad et al., 2015). Similarly in our study PBZ application resulted in compact plants and more chlorophyll content of leaves in irises. There was significant ($P \leq 0.01$) difference for flower diameter of 'Blue Magic' iris. The smallest flower diameters were 38.83 mm from 'Blue Magic' iris treated with 30 ppm PBZ, while 'Blue Magic' control was 99.63 mm. There was no difference for flower diameter of 'Frans Hals' iris cultivar (Table 3). Flower diameter of 'Frans Hals' treated with 30 ppm paclobutrazol was 109.1 mm, control

plants of this cultivar was 112.2 mm. PBZ treatments decreased the flower diameter of 'Blue Magic' cultivar because of the incomplete opening of the flower (Figure 5). Probably gibberellin inhibitor of paclobutrazol inhibited the elongation of pedicel+ovary in 'Blue Magic' iris. Çelikel and van Doorn (2015) reported that there is a relationship between flower opening and elongation of the pedicel+ovary in 'Blue Magic' iris since sheath leaves pose a mechanical barrier for flower opening (Çelikel and van Doorn, 2012). In our study paclobutrazol decreseased pedicel+ovary elongation, therefore flowers of 'Blue Magic' iris could't open completely. This resulted in a decrease of flower diameter in 'Blue Magic' iris. It was reported that the tepal size and pedicel length of plants applied flurprimidol were smaller than the control plants in 'Mona Lisa' lily cultivar (Pobudkiewicz and Treder, 2006). Flurprimidol application reduced the inflorescence and flower diameter of *Ornithogalum saundersiae* (Salachana and Zawadzińska, 2013). Uniconazole reduced the flower size by reducing peduncle, sepal and peduncle length of Fuchsia x Hybrida (Kim, 1995).

Table 3**.** The effects of paclobutrazol (PBZ) on chlorophyll content (CCI) in leaves and flower diameter of *Iris x hollandica* cv. 'Frans Hals' and *Iris x hollandica* cv. 'Blue Magic'

Mean ± Standard Error ($\overline{X} \pm S\overline{x}$)

Cultivar	Treatments	Chlorophyll content (CCI)	Flower diameter (mm)
'Frans Hals'	Control	28.80 ± 1.92 d	112.2 ± 2.12 a
	15 ppm PBZ	46.17 ± 1.73 c	110.0 ± 2.43 a
	30 ppm PBZ	52.87 ± 1.72 ab	109.1 ± 1.44 a
'Blue Magic'	Control	32.70 ± 1.58 d	99.63 ± 2.35 b
	15 ppm PBZ	51.26 ± 2.06 bc	43.60 ± 2.16 c
	30 ppm PBZ	57.00 ± 1.37 a	38.83 ± 1.94 d
Significance		0.000	0.000
Means of cultivars	'Frans Hals'	42.61 ± 1.85	110.43 ±1.93
	'Blue Magic'	49.99 ± 2.02	60.69 ± 2.04
Significance		0.000	0.000

* Different letters in the same columns indicate differences among treatments according to Duncan multiple range test (1%).

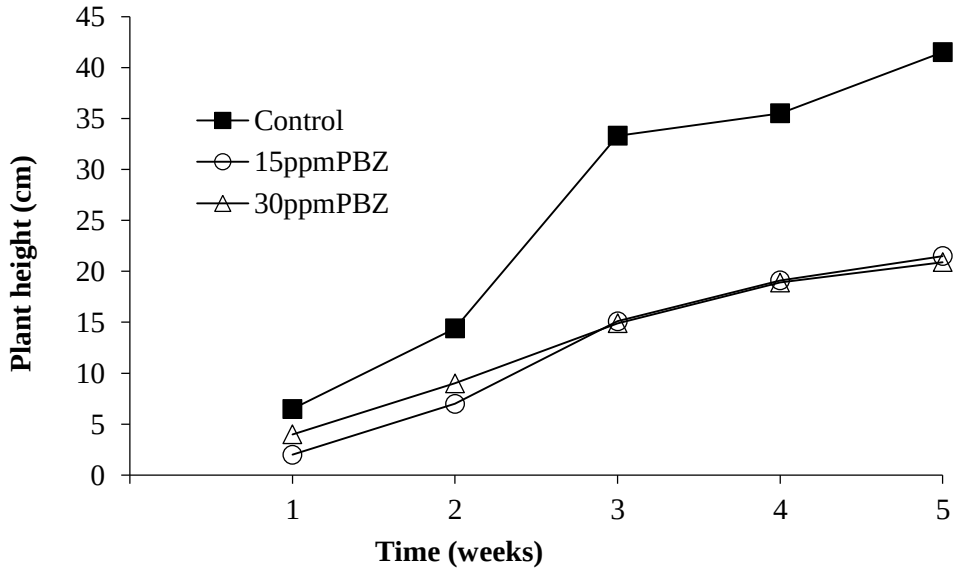


Figure 1. Effect of paclobutrazol (PBZ) treatment on plant height of *Iris x hollandica* cv. 'Frans Hals' during greenhouse production period. The plant height was started to measure 172 days after planting (1. week)

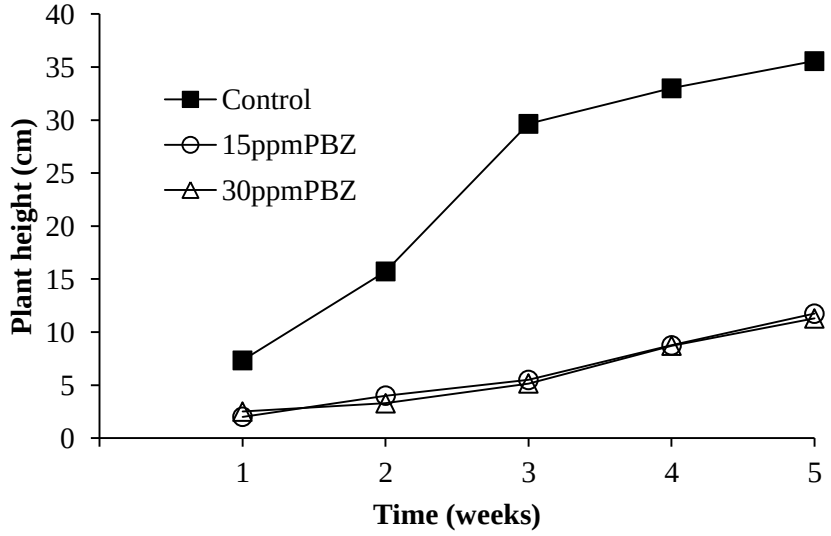


Figure 2. Effect of paclobutrazol (PBZ) treatment on plant height of Iris x hollandica cv. 'Blue Magic' during greenhouse production period. The plant height was started to measure 172 days after planting (1. week)

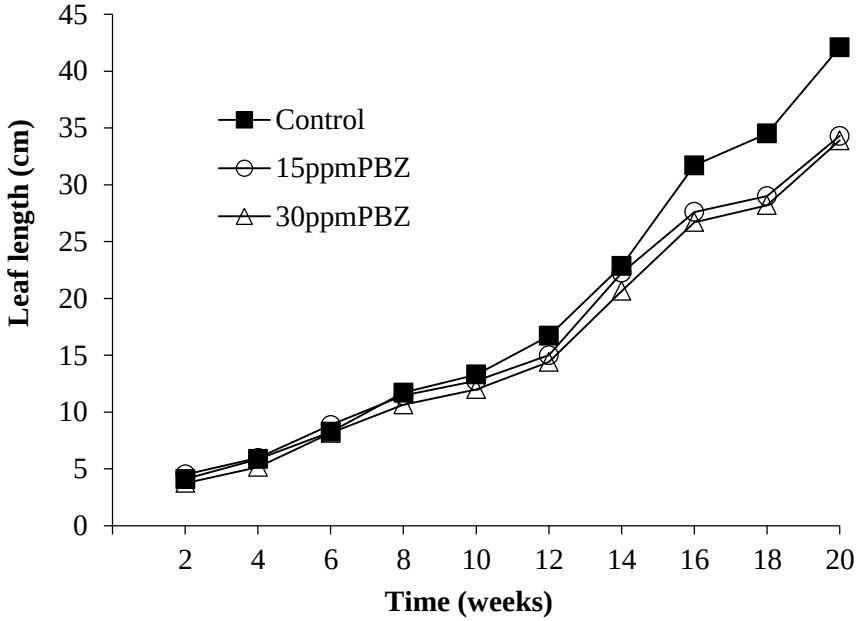


Figure 3. Effect of paclobutrazol (PBZ) treatment on leaf length of Iris x hollandica cv. 'Frans Hals' during greenhouse production period. The leaf length was started to measure 37 days after planting (1. week)

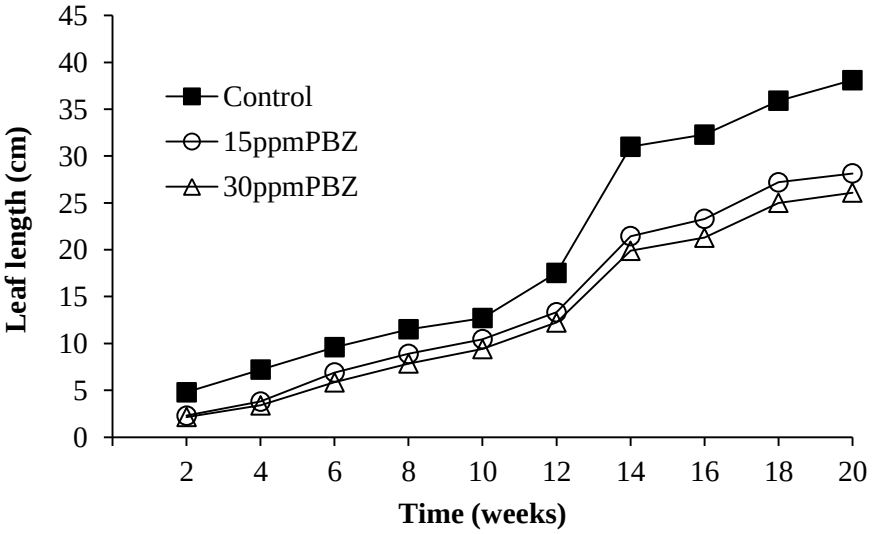
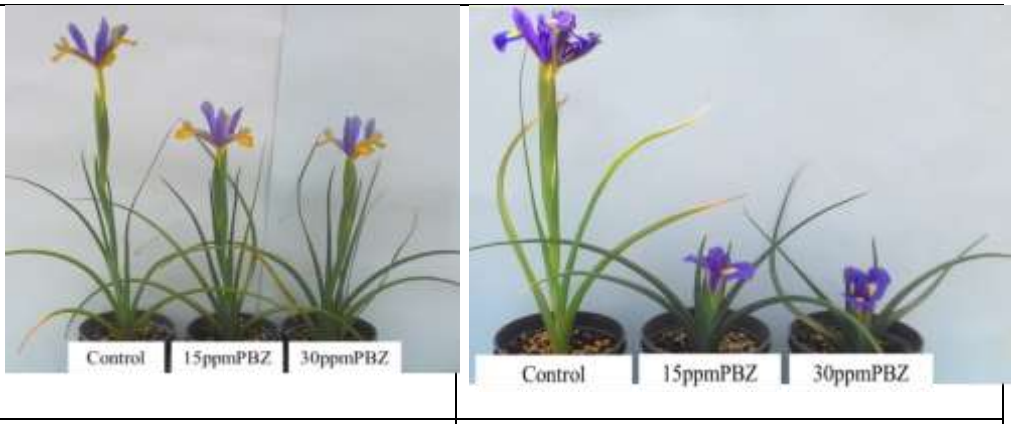


Figure 4. Effect of paclobutrazol (PBZ) treatment on leaf length of Iris x hollandica cv. 'Blue Magic' during greenhouse production period. The leaf length was started to measure 37 days after planting (1. week)



Iris x hollandica cv. 'Frans Hals'	*Iris x hollandica* cv. 'Blue Magic'

Figure 5. The effects of paclobutrazol (PB) bulb soak on *Iris x hollandica* cv. 'Frans Hals' and *Iris x hollandica* cv. 'Blue Magic'

CONCLUSIONS

Our results clearly indicated that plant growth regulators of paclobutrazol application controlled the plant height. Paclobutrazol shortened the leaf length and increased the chlorophyll content of leaves without shortening the flower life in iris cultivars. We found that paclobutrazol effectively controlled the plant height and there was no significant difference between doses. Therefore we suggest that the lower dose of paclobutrazol for 'Frans Hals' cultivar. However, paclobutrazol treatments caused excessive shortening of plant height in 'Blue Magic' cultivar which prevent the flower opening. Therefore we suggest that lower concentration of PBZ (<15 ppm) should be investigated for 'Blue Magic' cultivar.

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TRANSITION FROM SURFACE TO DRIP IRRIGATION IN MOROCCO: ANALYSIS THROUGH THE MULTI-LEVEL PERSPECTIVE

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ABSTRACT

Agriculture uses more than 80% of water resources in Morocco. The sector is inefficient in terms of water use due to the dominance of surface irrigation. To address this issue, there have been efforts in Moroccan strategies to convert surface irrigation to localized one. This paper analyses the dynamics of conversion from surface irrigation to drip irrigation in Fez-Meknes region (north-eastern Morocco) through the lens of the Multi-Level Perspective (MLP) on socio-technical transitions. MLP framework suggests that transitions are the results of dialectic interactions among a niche (cf. novelty of drip irrigation), a regime (cf. traditional system of surface irrigation) and the socio-technical landscape (e.g. policies). MLP was complemented with a multi-capital approach to better assess transition impacts. Results show that the area equipped with drip irrigation in Fez-Meknes region increased from 2174 ha in 2008 to 39290 ha in 2016. Different programs have been implemented in the framework of the Green Morocco Plan to foster irrigation transition e.g. the National Irrigation Water Saving Program (PNEEI), launched in 2007, aims to convert 550,000 ha to localized irrigation (e.g. drip irrigation) in 15 years. Thanks to these programs, financial and technical support has been provided to farmers to promote the adoption of water-saving irrigation techniques and practices. Farm-level results show that transition to localized irrigation decreases irrigation water use, increases yields and profitability (cf. gross margin per ha), and improves water productivity. Despite an enabling policy landscape and positive transition impacts, surface irrigation is still maintained in the region and farmers are reluctant to change for many reasons (e.g. age and education level, unclear land tenure, financial and administrative difficulties). Efforts are still needed to train farmers on irrigation scheduling and on the use of smart irrigation techniques to save water. Further research is required to better

understand current bottlenecks in the irrigation transition process and design appropriate and context-specific transition governance strategies.

Keywords: *Sustainable agriculture, Irrigation, Multi-Level Perspective, Sustainability transitions, Multi-capital model.*

INTRODUCTION

The agricultural sector is one of the pillars of the Morocco's economic and social development. The indicators on employment, value added, food self-sufficiency and foreign trade show the decisive position occupied by this sector in the national economy (e.g. Doukkali et al., 2003; HCP, 2017; MAPMDREF, 2018). Agriculture plays a crucial role in rural employment. Rural population accounts for nearly 45% of Morocco's population. Direct employment in agriculture alone accounts for 80% of rural employment and almost 50% of employment at the national level. In some areas, agriculture accounts for between 80% and 100% of rural income (HCP, 2017). The agricultural sector still contributes significantly to the gross domestic product (GDP). Depending on annual climatic fluctuations, this contribution is between 12 and 24%. In retrospect, the share of agricultural value added in the GDP decreased from 23% on average in the 1960s to 17% in the 1990s. In absolute terms, however, the agricultural value added nearly doubled between the 1970s and the 1990s; from 10.5 billion to 19 billion in constant Moroccan Dirhams (MAD) of 1980. The irrigated agriculture sector, whose contribution to agricultural value added averages around 45%, is a real catalyst for the national economy and plays a key buffer role especially during drought periods (MAPMDREF, 2018). Food self-sufficiency is a fundamental objective of agricultural policy in Morocco. Thanks to irrigated agriculture, among others, the country covers a large part of its basic food needs, despite the continued increase in domestic demand and changing climate. Moreover, the agricultural sector plays an important role in national foreign trade. Agricultural exports account on average for nearly 18% of exports value. Irrigated agriculture provides, on average, 75% of agricultural exports (MAPMDREF, 2018). Despite all the above-mentioned positive impacts generated by agriculture in Morocco, this sector remains the highest consumer of the scarce water resources; about 80% of water resources in the country is used in irrigation (DRH, 2016). The inefficiency of irrigation water use is due to the dominance of surface irrigation. Conscious of the problem of the management and saving of water, the public authorities have invested considerably for a better valorisation of the country's limited water resources through the Green Morocco Plan (PMV) and the National Irrigation Water Saving Program (PNEEI), which have encouraged farmers to convert from surface irrigation to localized irrigation. In order to understand this ongoing transition, this article analyses the dynamics of conversion from surface irrigation to localized irrigation systems in Fez-Meknes region (north-eastern Morocco) through the Multi-Level Perspective on socio-technical transitions (MLP) and to assess the impacts of this transition with a multi-capital approach.

MATERIAL AND METHODS

The present paper is based on a combination of secondary and primary data. Primary data were collected through a questionnaire with 76 farmers to assess the impacts of the transition from surface to localized irrigation. Secondary data were obtained from different sources such as the Provincial Agricultural Department in Meknes and El Hajeb concerning all information related to the conversion from surface irrigation to localized irrigation within the region (areas, subsidy procedures, etc.).

The study was conducted in Fez-Meknes region located in the Saïss plain (Figure 1). This plain covers 220,000 ha and is characterized by a high agricultural potential due to the presence of fertile soils and medium-depth underground aquifers (Berriane, 2002). The provinces of Meknes and El Hajeb were chosen as a study site. This choice is justified, on the one hand, by the average annual decrease of the piezometric level of the groundwater estimated at 3 m (Faysse et al., 2012; Quarouch et al., 2014) explaining the huge water consumption. On the other hand, this choice is also based on the evolution of areas equipped with drip irrigation especially that many farmers have benefited from the subsidies to convert from surface to drip irrigation. The choice of the studied crops (i.e. onion, potato, peach/nectarine and plum) was made based on the dominant cropping pattern as well as on the high crop water requirements in the study area.

In the context of the present study, conversion from surface irrigation to localized irrigation is considered as an example of transition. The analysis encompasses transition dynamics and transition impacts. The analysis of transition dynamics was carried out according to the analytical framework, based on the Multi-Level Perspective, proposed by El Bilali and Probst (2017). For this case study, the niche is drip irrigation system, the dominant system (cf. regime) is surface irrigation system and the landscape encompasses all water policies developed by the public authorities. As regards the determination of the type of transition, reference is made to the method developed by Geels and Schot (2007) while for the identification of the current transition phase the S curve of transition suggested by Rotmans et al. (2001) was used.

Figure 7. Location of Fez-Meknes region.

For the assessment of the impacts of conversion from surface system to drip irrigation system, a multi-capital model developed by Garrabé (2008) was followed to see the interaction between the different forms of economic, natural, human, social and institutional capitals (Garrabé et al., 2012). Given the problematic of the valuation of water resources, the paper zooms on the natural capital by considering three parameters namely irrigation water consumption, irrigation water use efficiency and water valuation (Bouaziz and Belabbes, 2002). Data on irrigation water consumption were collected through interviews with farmers and/or by making direct measurements within farms. The water use efficiency (WUE) was calculated dividing the obtained yield on the provided water quantity: *WUE = Yield (kg/ha) / Water used for irrigation (m^3/ha)*

The objective is to define how many kilograms can be produced by one cubic meter of water. The water valuation explains how much benefit a cubic meter of the provided water can generate in Moroccan dirhams (MAD).

The field study was carried out through a random and simple sampling of 76 agricultural farms sharing the two irrigation techniques, spread over 15 rural communes: Boufakrane (5 farms), Oued Jdid (4), M'haya (7), Majjate (8), Sidi Slimane Moul Al Kifane (4), Ait Ouallal (3), Ait Naamane (4), Sbaa Aiyoun (8), Ain Taoujdate (7), Ait Boubidmane (5), Bitit (6), Laqsir (4), Ait Yaazem (4), Jahjouh (4) and Ras Ijerri (3). The field study focused on the aspects relating to production systems, crop rotations, crop water consumption, irrigation frequency, operating expenses by crop, crop yields and prices by crop.

RESULTS AND DISCUSSION

The area of localized irrigation *niche* has been growing steadily in Fez-Meknes region for a decade (Figure 2), especially since 2008 with the launch of the Green Morocco Plan (PMV). The incentive scheme can cover up to all the costs of localized irrigation for farms of less than 5 ha and 80% for farms over 5 ha. In the context of PMV, the objective of area converted to localized irrigation is set at 550,000 ha by 2020. Compared to this objective, nearly 483,000 ha have already been achieved i.e. 88% of the target (MAPMDREF, 2018). As for *transition phase*, the S curve of transition proposed by Rotmans et al. (2001) suggests that irrigation transition in Fez-Meknes region has reached the acceleration / breakthrough phase. The period before the launch of PMV can be considered as pre-development phase while the launch of the Green Morocco Plan represents the moment of take-off. While Rotmans et al. (2001) considers that during the acceleration phase 'visible structural changes take place through an accumulation of socio-cultural, economic, ecological and institutional changes that react to each other', it seems that institutional/political changes have been predominant in the irrigation transition process and it is not clear whether the socio-cultural changes that took place are enough (in terms of speed and direction) to accommodate them. The pattern of transition shown in the curve is far from being typical as, surprisingly, the period of pre-development seems rather short. This – combined with the features of the acceleration phase described above – can have long-term implications in terms of the sustainability of transition as farmers had short time for learning about and adapting their farming system management to drip irrigation. At the end of the day, drip irrigation represents an innovation for farmers in the region, which implies that its introduction brings about some changes (cf. embedding processes) in the whole farm management system.

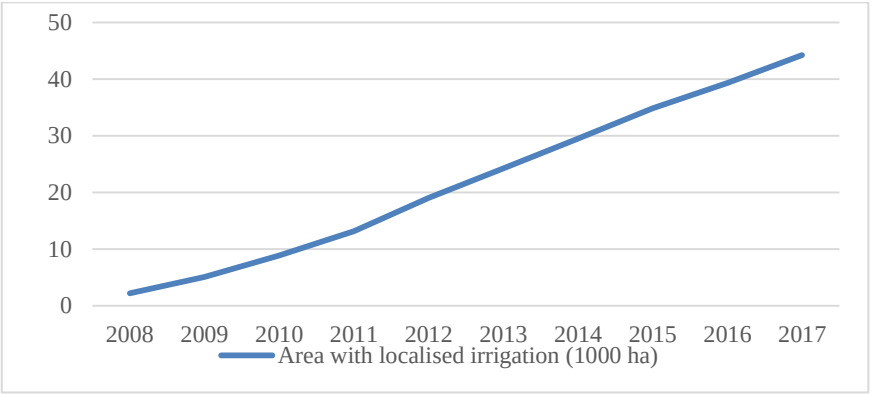

Figure 8. Evolution of cumulative area with drip irrigation in Fez-Meknes region from 2008 to 2016.

Source: Authors' elaboration based on data from the Provincial Agricultural Department in Meknes (2018).

Transition pathways suggested by Geels and Schot (2007) are derived mainly from sustainability transitions literature focused on energy and transportation sectors, which may suggest that they are not completely fit-for-purpose to describe transition processes in other sectors (e.g. agriculture, water). That being said, the pathway that seems more adapted to describe the conversion to drip irrigation is 'technological substitution'. In fact, public institutions in Morocco (cf. landscape) put forward a huge incentive system at a moment when the niche (i.e. drip irrigation) was sufficiently developed, the latter has broken through and replaced, to large extent, the existing regime (cf. surface irrigation). Drip irrigation is a technology that consists on the installation of an underground piping system and also apparent pipes equipped with water distributors (drippers). The water under pressure can be distributed all over the covered area within the farm and provided in the vicinity of the plant. The particularity of the system is the use of low flow rates and low water quantity with a high irrigation frequency leading to an important water saving, conditioned by a mastery of this technique. In contrary, surface irrigation is based on the land gravity, where the water can be distributed from the upstream (highest altitude point) to downstream (lower altitude point) within the farm leading to water losses by percolation and evaporation. Nevertheless, the pattern of transition accommodates also other transition pathways such as 'reconfiguration pathway'. In fact, it is worth nothing that drip irrigation innovation was adopted by farmers in Fez-Meknes region to solve local problems (cf. increasing water scarcity) and such adoption, subsequently, triggered further adjustments not only in irrigation systems and farm management but also in regional agriculture.

In the context of irrigation development in Morocco, irrigated land has grown from about 150,000 ha in 1960 to nearly 1.6 million ha in 2010 (Alloussi and Anbari, 2012). The socio-technical *landscape* in Morocco is largely favourable for irrigation transition. As part of PMV, the country considers adaptation to climate change a priority, particularly through water saving, with a target of 1.4 billion cubic meters of water savings per year. These savings would allow the extension of the irrigated area by 550,000 ha without increasing the irrigation water use amount (MAPMDREF, 2018). Water is at the heart of the transversal reforms of the Green Morocco Plan, through: developing means of mobilization of conventional and unconventional water resources; valuation of water resources allocated to irrigation; establishing a highly incentive pricing for water saving and valuation; promoting a policy of voluntary management of agricultural water demand through, inter alia, activation of a true water police, generalization of water-saving irrigation techniques. For that, various specific programs have been put in place (Boularbah et al., 2107):

- The National Irrigation Water Saving Program (PNEEI): It aims to convert 555,000 ha to localized irrigation over 15 years (2007 – 2022) with a budget of 37 billion MAD.
- Irrigation Extension Program: It aims to extend irrigated area in Morocco by 155,000 ha over 12 years (2008 – 2020) with a budget of 19.5 billion MAD.

- Public-Private Partnerships (PPP) in irrigation: They aim to improve the quality of irrigation services and to ensure the sustainability of irrigation infrastructure in irrigation schemes through the active participation of water users and the private sector.

Three major motivations are put forward to promote drip irrigation in Morocco: (i) labour saving and reduction of workload through irrigation system automation (drip irrigation monitoring requires less manpower and automation of watering of the different farm plots facilitates work organization); ii) improvement of agricultural products quality and quantity (this is ensured by the rationalization of the inputs of water and fertilizers and a better intra-plot distribution with consequent improvement of yields); and iii) the saving of water irrigation. Despite all its advantages, there is an incomplete transition as some farmers are still reluctant to transition to localized irrigation systems. Many farmers in the survey sample would like to convert to localized irrigation but various constraints hamper that. The principal ones relate to administrative complexity and bureaucracy, land tenure and financing. The problem of financing is mentioned as the main reason for the non-conversion. The farmers declare they had the intention of converting their irrigation system to localized irrigation, but the lack of financial means and the difficulty of obtaining credit from the suppliers of irrigation equipment, while being forced to anticipate almost 50% of the drip irrigation system cost, prevent them from realizing it. Some other farmers filed their grant applications but did not follow up them claiming that the procedure is complex and that obtaining the subsidy is not guaranteed as it is not released until after drip irrigation project implementation. Another problem mentioned by some farmers is that of land status due, among others, to the Islamic inheritance rules. They argue that since there is co-ownership, the realization of the conversion project requires the agreement of all the coheirs, which arises problems regarding the multiplicity of decision-makers and even of lack of trust. They state that the co-heirs do not want the farm to be entrusted, by proxy, to only one of them.

The analysis of *transition impacts* was performed at farm level and focused on the economics of natural capital (cf. valorisation of irrigation water). The two irrigation systems were compared by farm type between the different crops chosen for three parameters, namely irrigation water consumption, water use efficiency and irrigation water valuation (Table 1). The results of this comparison show that transition in the study area generates the following impacts: a decrease in irrigation water use by 36%, 46%, 59% and 61% for onion, potato, peach/nectarine and plum, respectively; a strong increase in the valuation of irrigation water by the different selected crops, by 208%, 151%, 83% and 431%, for onion, potato, peach/nectarine and plum, respectively. With regard to profitability, the shift to localized irrigation would allow an increase of yields of the studied crops of about 49% for onion, 29% for potato, 12% for peach/nectarine and 34% for plum. In terms of profitability, this transition would lead to an increase in the gross margin per hectare of the four crops, by 44% for onion, 23% for potato, 25% for peach/nectarine and 41% for plum.

Table 1. Parameters for irrigation water valuation by crop, irrigation system and farm category.

Parameter		Onion			Potato			Peach/Nectarine			Plum		
Irrigation water consumption (1000m^3/ ha)	FC	SF	MF	LF	SF	MF	LF	SF	MF	LF	SF	MF	LF
	SI	11.9	12.7	11.1	13.5	11.9	12.7	17.3	15.3	13.9	15.9	17.3	18.7
	DI	8.1	5.4	9	7.5	6.8	6	6.2	6.1	6.4	6.1	6.6	6.3
Water Use Efficiency (kg/m^3)	FC	SF	MF	LF	SF	MF	LF	SF	MF	LF	SF	MF	LF
	SI	3.03	3.3	4.1	2.01	2.6	2.9	1.04	1.3	1.9	1.07	1.2	1.2
	DI	6.7	11.1	7.8	4.9	5.9	7.5	3.2	3.9	4.4	2.9	4.8	4.8
Valuation of irrigation water (MAD/m^3)	FC	SF	MF	LF	SF	MF	LF	SF	MF	LF	SF	MF	LF
	SI	1.5	1.6	2.1	1.7	2.5	3.2	1.3	2.2	4	0.8	1.3	1.3
	DI	3.7	7.4	4.5	4.5	5.7	8.3	2.1	4.2	7.9	2.4	6.7	10.1

Legend:

- Farm Category (FC): SF: Small Farms with less than 5 ha of utilized agricultural area (UAA); MF: Medium-sized Farms with an UAA between 5 and 15 ha; LF: Large Farms with an UAA larger than 15 ha.
- Irrigation system: SI: Surface irrigation; DI: Drip Irrigation.

Source: Authors' elaboration based on field survey results.

CONCLUSIONS

This study confirms the crucial role played by public policies in sustainability transitions. In fact, agricultural and water saving policies (e.g. PMV, PNEEI) have, to a large extent, shaped the contours of irrigation transition in Morocco. Nevertheless, while public policies are necessary and justified (given the positive natural, social and economic impacts of irrigation transition), the present study also shows that they are insufficient. Despite massive investment of the country in conversion to localized irrigation, the transition is incomplete and one can talk of a 'transition-in-the-making'. In fact, surface irrigation still persists and the two irrigation systems coexist in Fez-Meknes region and, sometimes, even at the same farm. This might be due to the fact that the opportunities/benefits created by the landscape for the niche (i.e. drip irrigation) are insufficient to encourage farmers and overcome their resistance to convert their farms given the difficulties related to the necessary learning as well as the viability of drip irrigation and the high cost of its installation. Therefore, it seems that only an incentive system is not enough to bring about the necessary genuine irrigation transition in Morocco and the public institutions should move towards a combination of incentive system for the drip irrigation niche with more pressure on the regime (i.e. surface irrigation); the latter can be done by, among others, changes in irrigation water pricing and more stringent rules regarding the use of surface irrigation especially in water-scarce regions of the kingdom and/or where water resources (both surface and underground) are depleted.

To the best of our knowledge, this is the first study that analyses irrigation transition in Morocco using MLP. The originality of the study also lies in the combination of MLP and a multi-capital approach. In doing so, the present study

also addresses in an effective way the weakness of MLP regarding the impacts of transition. In fact, while MLP seems appropriate in analysing the dynamics of transition it falls short when it comes to the assessment of transition impacts.

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MECHANISMS AND ECONOMIC CONSEQUENCES OF PUBLIC INTERVENTION IN AGRICULTURAL HOLDINGS IN POLAND DURING THE PERIOD OF EU MEMBERSHIP

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ABSTRACT

The concept of public intervention in agriculture can be understood very broadly, as any act or omission of the operation of public state institutions. The article discusses the economic reasons for the public intervention, then presents the types and effects of intervention implemented by the CAP as well as the impact of intervention policy on economic decisions of agricultural producers. In the further part of the article, based on the statistic data from 2004-2016, were presented the changes in agriculture sector. A characteristic feature of this process is the deagrarisation the national economy and the development of rural areas. Throughout this process, it is extremely important for the ongoing structural changes to result in the improvement of the competitive position of farms and long-term and sustainable rural development. Poland's accession to the EU has generated new economic and organisational conditions to support structural changes in the broadly defined food economy and rural areas. Policy instruments implemented within the CAP create chances for the stabilisation of structural policy conditions over the period of several production cycles, thus stimulating the desired changes in the area structure of farms, the improvements in the competitiveness of production, environmental protection and multi-functional development of rural areas. Thus they are a fundamental instrument supporting the process of modernisation of Polish rural areas and agriculture. The article conclusions refer to structural changes and to competitiveness of agri-food sector.

Keywords: *public support policy, competitiveness, intervention in agriculture.*

INTRODUCTION

The active role of government and justification for state intervention in economic process results from the conviction about market failure (Bator, 1958). This concept suggests that in the realities of the market economy the processes of allocation of goods and services show a number of frictions. As a result, the state of actual equilibrium achieved by the market is not compliant with Pareto optimum. In broader terms, the concept of market failure identifies the scope and

circumstances of observed defects of market mechanisms that lead to the perpetuation of market imbalances (Baumol, 1952). In this context, it emphasizes the positive aspects of market intervention by public authorities (Stiglitz, 1989). Economic theory distinguishes a number of reasons for market failure. The mechanism and the logic of intervention in modern agriculture is shown in figure 1. Arrow (1983) was one of the first to point out that, in fact, one can distinguish two different states of efficiency in the allocation of goods depending on the degree of fulfilment of the Pareto demands. The first approach suggests that each allocation of goods in equilibrium meets only the demand of the so-called "poor efficiency" in the sense of Pareto.

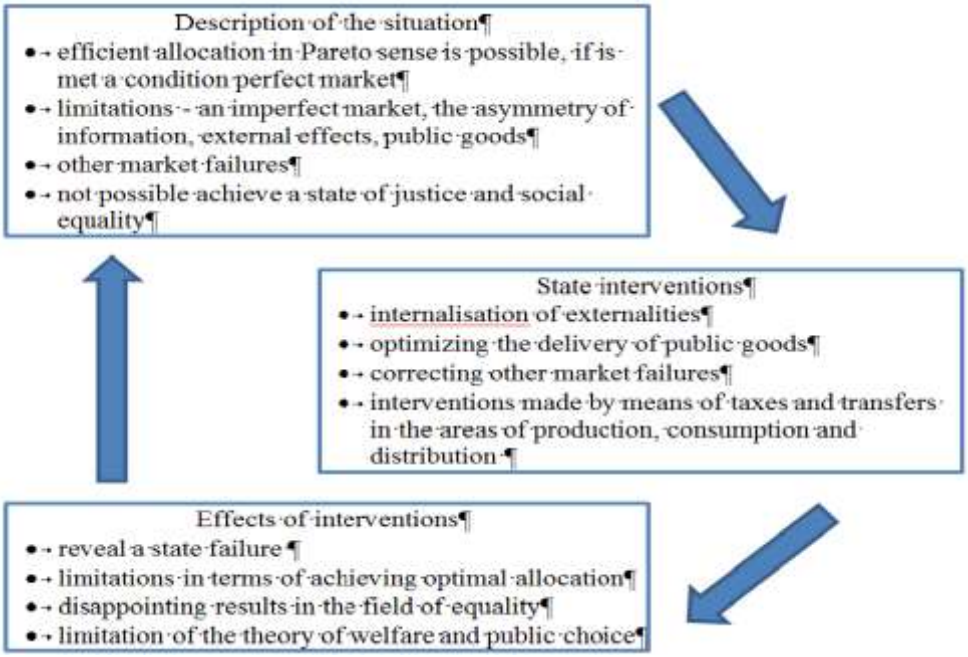

Figure 1 The mechanism of the modern intervention in agriculture

Source: Own study based on a literature review of studies of welfare economics and public choice economics

The global experiences prove that the market and the state have to co-exist and the state intervention should be always limited to support market mechanism and not replacement thereof. The state should interfere only when it has a clear advantage over the market mechanism; hence only when the market fails to protect the general interests of the society (Woś, 2004). The contemporary global economy often rejects the thesis on the perfect market (Czyżewski, 2007) thereby justifying the role of state intervention. When explaining the main reasons for intervention in the modern global agriculture point to the high level of risk linked to agricultural activity and lack of effectiveness in prevention of this risk. The risk results from e.g. changing climate conditions, lack of sufficient information and

underdevelopment of agribusiness structures, including also consultancy. The need for interventions in the agribusiness sector is justified also by: the phenomena of external costs and effects, low price elasticity of supply, lower level of labour productivity than in other sectors of the national economy, low mobility of the workforce employed in agriculture, the need to provide public goods, implementation of the sustainable development concept.

Implementing the objectives of CAP support has important impacts on food economy. The current objective set of the CAP, according to the "EU 2020" strategy, is that agriculture should contribute to smart, sustainable and inclusive growth. Government policy measures have static effects, risk-related effects and dynamic effects on production, and different transfer efficiency on farm income depending on policy tools applied. The impact of agricultural subsidies on income distributional effects depends on their type, the structure of the markets and the existence of market imperfections. Most of the studies investigate the direct impacts of subsidies on prices, output, income, the environment, etc. by assuming that subsidies do not alter the structure of agricultural markets and do not interact with market institutions. In reality, government policies may have various unintended effects (they can change the structure of market organization or crowed out some market institutions) (Forgasi et al., 2014). The objective of this study is presentation of the mechanisms and economic consequences of public intervention in agriculture in Poland during the period of EU membership which seems to be very important for rural economy as well as whole economy of Poland.

MATERIAL AND METHODS

The basis for the research was studied literature, especially in the field of main stream economics, welfare economy and public choice theory, referring to the question and to the consequence of public support in agriculture. Documentation studies were carried out also in the field of literature consolidating issues related to public support and process of structural changes, innovation transfer, the development strategy of determining the directions of the policy and the main acts forming the regulatory environment. Assessment of the importance of structural changes in agriculture was carried out using methods of descriptive and comparative analysis. The empirical material was the statistical data of the Central Statistical Office (CSO) for the years 2004-2016 and Agency for Restructuring and Modernisation of Agriculture (ARMA).

RESULTS AND DISCUSSION

The specific nature of the agricultural sector and its entities boils down primarily to the limited mobility of production factors involved in this sector. This particularly regards land, family labour resources, and, to a significant extent, the capital adjusted to agricultural activity. Agriculture and its entities are deprived of the benefits from transferring resources to more effective sectors, which determines the nature of competitiveness. Following the accession to the EU, there have been major changes in the agrarian structure, which continued long-term trends. In the

period preceding accession to the EU (1996-2002), large agricultural holdings (20-50 ha of utilised agricultural area - UAA), which took over arable land from small and medium agricultural holdings, but also from holdings of over 50 ha, developed dynamically. The growth in numbers was accompanied with the decrease of surface. The arable land of holdings below 1 ha grew, but their number dwindled. Following the accession to the EU, the number of holdings decreased by 28%, and their surface – by 3%. The development of large holdings lost dynamics, but still arable land was taken over by holdings of the surface of 20 to 50 ha, for both smaller holdings, of which the number and area decreased, and bigger, the number of which, despite the decrease of the area, grew. The number of holdings taking over land slightly increased. The number of holdings smaller than 1 ha decreased by 27%. To a slightly lesser extent the number of small and medium holdings decreased (from 25% to 16%). Their area also shrunk, including, to a largest extent, in the group of 10 to 20 ha (by 8%). These changes indicate that the Polish agriculture, despite major changes, is still to a large extent dispersed. Figure 2 shows the use of land in different groups of farms in 2016. The basic source of reduction in production cost is the change to the relation between production factors – particularly the relation between capital on one hand, and land and labour on the other. In Poland, the equipment of labour with land and capital is much lower than in most EU countries, which determines relatively low labour and land productivity (Figure 3). Low cost of labour in Poland is the primary factor that contributes to the advantage of Polish agriculture over agricultural products of the majority of EU countries with regard to cost and prices. Low remuneration for labour, however, cannot be the basis for maintaining competitiveness of agri-food sector product on the European and global market. Improvement in efficiency of use of land and labour means also the improvement in the quality of production conditioned by technical and technological progress, and the level of producers' knowledge. At the same time, the land cultivation system, which is less intensive in Poland than in most EU countries, is a kind of competitive advantage because Polish agriculture can become "sustainable" agriculture faster that agriculture of other EU countries, which is the EU objective in the 2050 perspective.

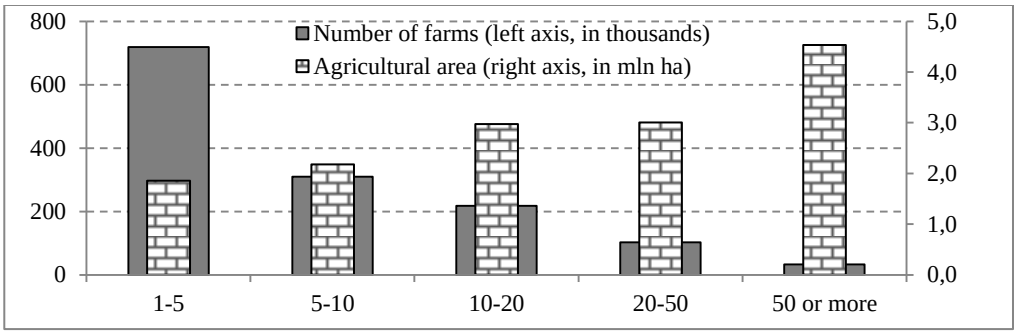

Figure 2. Land use by groups of farms in 2016.
Source: Agriculture in 2016. CSO data, Warsaw 2017.

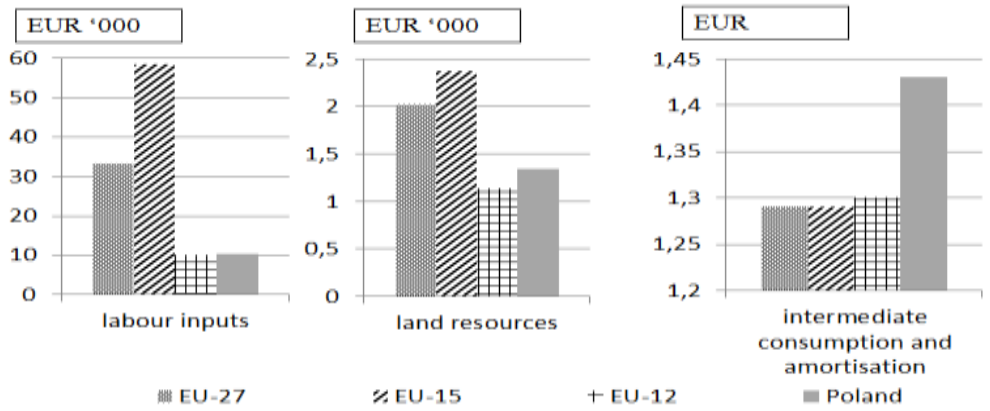

Figure 3. Productivity in Poland against average values for groups of EU Member States in 2016.

Source: Eurostat 2017.

The EU funds had a significant share in the financing of transformations in agriculture until Poland's accession to the EU. The direct payments are the most common type of support, each year about 1.4 million of farmers use this form of support. The value of payments in the 2004-2016 period increased and fluctued betwen ca. Euro 1.5 billion to Euro 3.5 billion per year. When calculated per one farm it reaches an average of ca. Euro 2.3 thousand, and this form of support is used by 87% of farms having an area of more than 1 ha. An equally important source of income (regardless of production, and only based on the farm's location) are payments for less-favoured areas (LFA). Each year these payments are granted to ca. 700 thousand farmers, i.e. half of those receiving direct payments. The land surface covered with LFA payments amounts to ca. 6.9 million ha. The manner of spending of the resources is not subject to settlement. Smaller farms usually allocate the granted payments to current needs and means of production (fuel, fertilisers), while the bigger ones also make investments.

The resources earmarked for investments are also an important source of aid for farms. So far, the financial resources for investments in farms, available under SAPARD, SOP "Agriculture", RDP 2004-2006, RDP 2007-2013 and RDP 2014-2020 were used in their entirety. The SAPARD programme was aimed at preparing the Polish agri-food sector to the accession, in particular in the adjustments to the sanitary, hygienic and environmental protection requirements of the EU. After 2004, the strategic objectives of agricultural policy implemented via SOP Programme "Agriculture" and RDP 2004-2006 covered: improving the competitiveness of the agri-food sector, sustainable development of rural areas, improvement of the condition of the natural environment, improvement of the quality of life and diversification of economy in rural areas. In the next programming period, the RDP 2007-2013 has become the programme to support the implementation of the concept of multifunctionality of agriculture and rural

development. It assumed economic strengthening of farms and an increase in the competitiveness of the agri-food sector, while assuring instruments for diversification of economic activities towards the acquisition and the creation of alternative sources of income for the rural population. RDP 2014-2020 for Poland focuses on three main objectives, i.e. supporting competitiveness and productivity in the agri-food sector, ensuring sustainable management of natural resources and climate action, as well as achieving sustainable territorial development of rural economies through the development of local infrastructure, investments in the field of education, culture and public services, creation of new and maintenance of existing jobs.

In the 2004-2016 period, the cumulative value of support for the agri-food sectors from three main sources of support: the payments from the EU budget, a grant from the national budget to KRUS and grant from the national budget, exceeded in total PLN 581 billion (EUR 145 billion) (Figure 4).

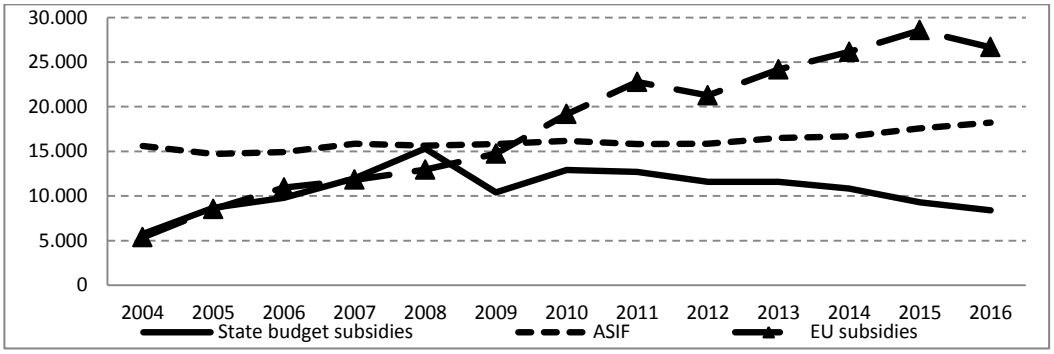

Figure 4 Budget spending on the agricultural sector in 2004-2016 (PLN '000 000).
Source: Authors' own calculation according to Budget Act (different years).

Rural development programmes, and indirectly also direct payments, are the primary source of support for investments in the Polish agriculture[2]. The role of the latter is particularly significant in farms with a large area. The area-related nature of direct and supplementary payments and LFAs[3] means that each year, farms receive cash they can spend on any purpose they want. The importance of these payments in the support for income, indirectly also for investment, is evidenced by the fact that they are received commonly. In order to obtain the payments, a farmer

[2] Direct payments in agriculture fulfil a number of functions, i.e. the income function (they compensate the farmers' increased production costs), the stimulating function (they can define the direction of agricultural production), the modernisation function (they can be used for co-financing of investment), the information function (they indicate the areas of production).

[3] About half of the land used for agricultural purposes in Poland is located within LFAs. These include e.g. the areas where there are difficult climatic conditions, rainfall is too big or too small, there is a disadvantageous topography (e.g. mountains), or the quality of soil is low.

is only required to properly fill in an application and keep land in a good agricultural condition.

CONCLUSIONS

In the last decade the structural changes taking place in the Polish agriculture became more dynamic. The most important among them cover: a drop in the number of farms with simultaneous growth in the share of the largest farms, which directly influences the increase in the average area of farms, drop in employment in agriculture and progressing production concentration and specialisation. The structural changes are, however, slow and cannot be efficiently accelerated due to non-agricultural circumstances. The Polish agriculture is still characterised by a strong polarization of the agrarian structure.

Policy instruments implemented within the CAP created chances for the stabilisation of structural policy conditions over the period of several production cycles, thus stimulating the desired changes in the area structure of farms, the improvements in the competitiveness of production and multi-functional development of rural areas. Thus they are a fundamental instrument supporting the process of modernisation of Polish rural areas and agriculture (Wigier, 2014a). Today, we already know that CAP has actually caused an increase in support for agriculture, while structural funds have triggered considerable cash flows intended for modernisation of food economy and rural areas development. However, not all investments proved to be effective, which undoubtedly prompts us to reflect on the scale and the value of investments in agriculture.

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If received significant help in designing, or carrying out the work, or received materials from someone who did a favour by supplying them, their assistance must be acknowledged. Acknowledgments are always brief and never flowery.

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